Addressing Micronutrient Malnutrition Through Enhancing the Nutritional Quality of Staple Foods: Principles, Perspectives and Knowledge Gaps

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Five years ago, with international funding, several international agricultural research centers set out to explore the potential to improve the micronutrient quality of some staple food crops. Five objectives were identified, and all needed a favorable result if breeding for higher micronutrient density in the staples were to be deemed feasible. Useful genetic variation to exploit was needed. The traits needed to be manageable in a breeding program (simple screening and high heritability), and stable across a wide range of environments if impact was to be large. Above all, the traits needed to be combinable with traits for high yield to ensure that farmers chose the improved lines. Finally, it was necessary to show that the new types actually improved the health of humans of low nutrient status representing the target populations. The extra nutrients needed to be bioavailable to the gut. Today, only this last essential criterion remains to be fully satisfied. All other criteria are met to levels that lead us to claim that breeding for nutritional quality is a viable, practicable, and cost-effective strategy to complement existing interventionist strategies. Subject to satisfying the last criterion, and results are encouraging, we call for a major funding initiative, and the installation of a new paradigm for 21st century agriculture: one espousing food systems that are highly productive, sustainable, and nutritious. This paper reviews the case for and the rationale behind the project that is underway, gives an overview of the results to date and looks at the critical issues that still remain to be confronted.

I. INTRODUCTION

What is agriculture to human existence? Agriculture provides the nutrients essential for human life. The reality of this is hidden when we use the less definitive term "food"; food may or may not provide all the necessary nutrients. If agriculture fails to produce adequate amounts of foods containing enough nutrients in balance to meet human needs, health will deteriorate, livelihoods will diminish, national morbidity and mortality rates will rise, development will stagnate or decline, discontent and civil unrest will swell, political upheaval will ensue and human suffering will dramatically increase. Insufficient output of even one essential nutrient over a long time will produce these dire consequences. Therefore, it is imperative that the world's agricultural institutions understand that the nutritional health of humans globally is largely dependent on the nutrient outputs that agricultural systems produce. In the twentieth century, agricultural institutions have not viewed themselves as suppliers of nutrients with an explicit goal to improving human nutrition and health. Such a view must be reached if we are to reduce malnutrition around the world, and prevent much human suffering resulting from the ever increasing demand on our food systems for nutrient resources brought on by the increasing population pressure.

The world's population has grown rapidly since 1950 swelling from 2.52 billion to over 6 billion today—an increase of nearly two and one-half times—because of dramatic reductions in mortality in populous developing countries (Fig. 1). While the population growth rate has diminished from its maximum of 2.0% per year during 1965–1970, the population is still growing at a rate of 1.3% per year, adding annually another 78 million mouths to feed. This growth in population is not expected to subside until well into the twenty-first century. Most of this

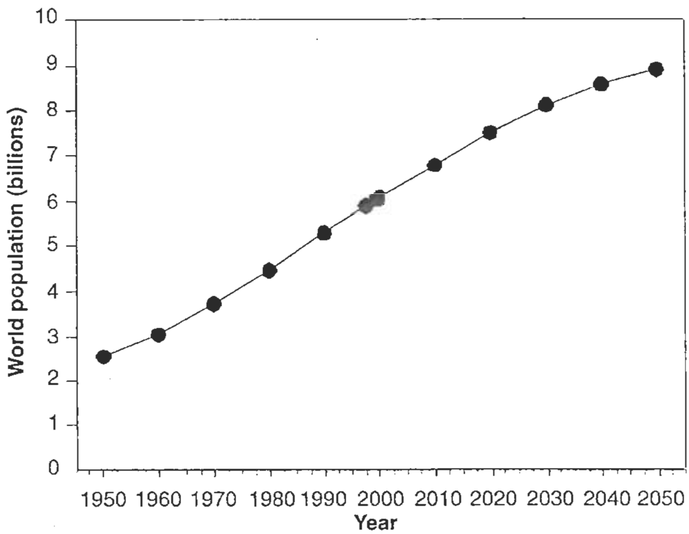

Figure 1 Trends in world population growth from 1950–2050. (Data from United Nations [forthcoming], World Population Prospects: The 1998 Revision, United Nations, New York).

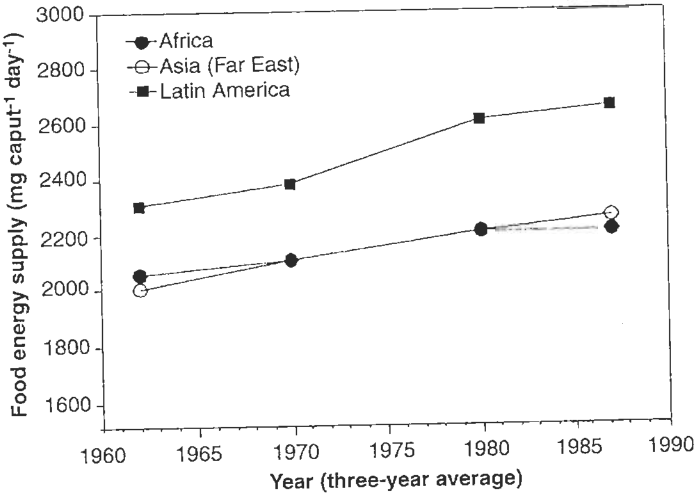

Figure 2 Changes in dietary energy (i.e., Kcalories capita^{-1} day^{-1}) supply from 1962–1987 for Africa, Asia (Far East) and Latin America. (Data from United Nations Administrative Committee on Coordination-Subcommittee on Nutrition, 1992). Average caloric requirement for an adult is assumed to be 2350 kcal^{-1} day^{-1} (Uvin, 1994).

increase in population will occur in developing nations, especially countries in South Asia, Africa, and Latin America. By 2050, the world's agricultural systems will need to generate enough food to support the nutrient requirements of over 8.9 billion people (United Nations, 1998). To avoid widespread famine, this will require that agriculture, once again, match its earlier success in increasing food production demonstrated during the "green revolution." By 2020, meeting the energy demand of an expanding population will require that farmers produce one and a half times as much food as they did in the early 1990s. This must all be accomplished with about the same land under cultivation but with dwindling water resources and declining soil fertility (Brown and Flavin, 1999; Pinstrup-Andersen, 1999). Growing sufficient food will not in itself assure adequate nutrition and healthy, productive lives for all.

Today, most agricultural systems in the developing world do not provide enough nutrients. Many fall short of supplying enough micronutrients (14 trace elements and 13 vitamins) to meet human needs, even though the production of energy and protein via cereal crops appears to be adequate to feed the world (Fig. 2, Welch *et al.*, 1997). However, the problems of poor food distribution means that over 800 million people do not receive enough energy (calories) and protein to meet their daily requirements (Table I) (Uvin, 1994). In 1995, of the estimated 10.4 million deaths among children less than 5-years-old, protein-energy malnutrition was a causative factor in 5.1 million of these deaths (World Health Organization, 1999).

Table I

Global Energy Deficit Regions Where Dietary Caloric Supplies (Between 1988 and 1990) Were Below Levels Required to Satisfy Human Needs

World Region	Number of countries	Population (millions)
Asia	4	262.4
Latin America	7	67.2
Near East & North Africa	1	12.5
North America, Australia, Western & Eastern Europe, and the Community of Independent States (former USSR)	0	0.0
Small islands	4	1.1
Sub-Saharan Africa	32	459.1
Total	**48**	**802.3**

Modified from Uvin (1994).

These figures, disturbing as they are, do not tell the full story: over half of the world's population does not consume enough of each micronutrient in their food to support good health. Tables II, III, and IV show the extent of the micronutrient deficiency problems worldwide for only three micronutrients—iron, iodine, and vitamin A. Other micronutrients, including zinc, selenium, folic acid, vitamin C, vitamin E, vitamin D, thiamin, vitamin B_{12}, and niacin, are almost certainly impairing the health and productivity of large numbers of people in the developing world, especially resource-poor women, infants, and children. However, for these nutrients there are no data available to quantify the extent of global deficiency because of the lack of reliable and affordable clinical tests (Maberly *et al.,* 1994; World Health Organization, 1999).

Even though micronutrients are needed in minute quantities (i.e., micrograms to milligrams per day), they have tremendous impact on human health and well being. Insufficient dietary intakes of these nutrients impair the functions of the brain, the immune and reproductive systems and energy metabolism. These deficiencies result in learning disabilities, reduced work capacity, serious illnesses, and death. Micronutrient malnutrition is a serious global affliction that limits the work capacity of people and seriously hinders economic development (Anonymous, 1994).

Economic theory predicts that with increased income, individuals should be able to purchase more food and diversify their diets, especially with animal products, thereby improving their micronutrient status. This does not appear to be the case. For example, in Asia and Latin America, the availability of iron in food has declined even though income (Anonymous, 1994) and the availability and intake of foods containing high amounts of energy (i.e., cereals) have risen significantly

Table II

Estimated Anemia, Iron Deficiency Anemia and Iron Deficiency Prevalences in Populations from Various Global Regions

WHO global regions	Populations Affected					
	Anemia		Iron deficiency anemia		Iron deficiency	
	Number (millions)	Prevalence (%)	Number (millions)	Prevalence (%)	Number (millions)	Prevalence (%)
Africa	233.7	38.8	175.3	29.1	438.2	72.8
Eastern Mediterranean	179.5	38.5	134.6	28.9	336.6	72.2
Europe	79.8	9.2	59.9	6.9	149.6	17.3
South-East Asia	765.2	52.7	573.9	39.5	1434.8	98.8
The Americas	141.7	18.1	106.3	13.6	265.7	33.9
Western Pacific	578.4	38.4	433.8	28.8	1084.5	72.0
Total	**1978.3**	**34.3**	**1483.7**	**25.7**	**3709.3**	**64.3**

Modified from WHO (1999).

(Figs. 2 and 3). At the same time, iron deficiency in women, infants, and children in resource-poor families has risen dramatically. Indeed, in South East Asia, iron deficiency now afflicts 98.2 % (over 1.4 billion) of the people in that region (Table II). Within the developing world, serious vitamin and trace element deficiencies

Table III

Prevalence and Number of People Affected with Iodine Deficiency Disorders (IDD), Including Goiter and Cretinism, in Various Global Regions

WHO global regions	Populations affected			
	Goiter		Cretins	
	Prevalence (%)	Numbers affected (millions)	Prevalence (%)	Numbers affected (millions)
Africa	23.7	147	1.48	4.21
Americas	6.5	52	0.33	0.34
Eastern Mediterranean	30.3	145	2.59	7.18
Europe	10.7	93	0.26	0.48
South-East Asia	14.9	220	0.81	3.56
Western Pacific	15.5	254	0.14	0.74
Total	**15.6**	**911**	**0.92**	**16.51**

Modified from WHO (1999).

Table IV

Vitamin A Deficiency Estimates in Populations of Children Under the Age of Five Years in Various Global Regions

	Affected Population			
	Subclinical Evidence		Clinical Signs	
WHO global regions	Number (millions)	Prevalence (%)	Number (millions)	Prevalence (%)
Africa	49	45.8	1.08	1.0
Eastern Mediterranean	23	31.5	0.16	0.3
Europe	—	—	—	—
South-East Asia	125	70.2	1.3	0.7
The Americas	17	21.5	0.06	0.1
Western Pacific	42	30.0	0.1	0.1
Total	**256**	**40.3**	**2.7**	**0.1**

Modified from WHO (1999).

persist and are not necessarily corrected by increased income within an acceptable period of time (Anonymous, 1994).

Dysfunction of the food system from low micronutrient output is affecting more

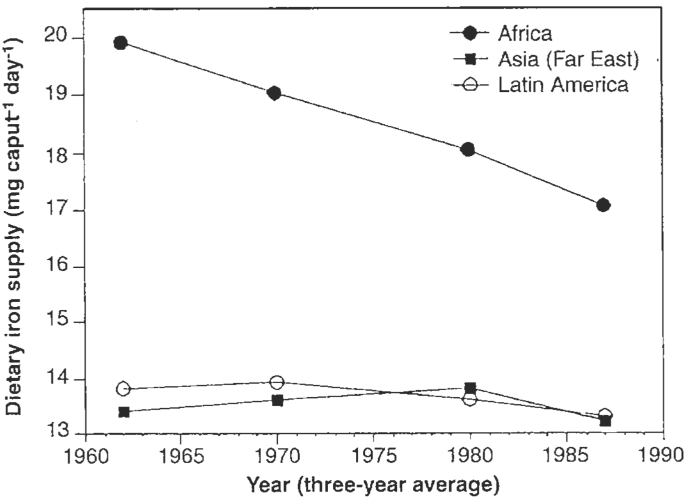

Figure 3 Changes in dietary iron (i.e., mg capita^{-1} day^{-1}) supply from 1975 to 1990 in Africa, Asia (Far East), and Latin America. Daily iron requirements for people in these regions vary from 19 and 24 mg Fe capita^{-1} day^{-1} depending on traditional diets consumed (data from United Nations Administrative Committee on Coordination-Subcommittee on Nutrition, 1992).

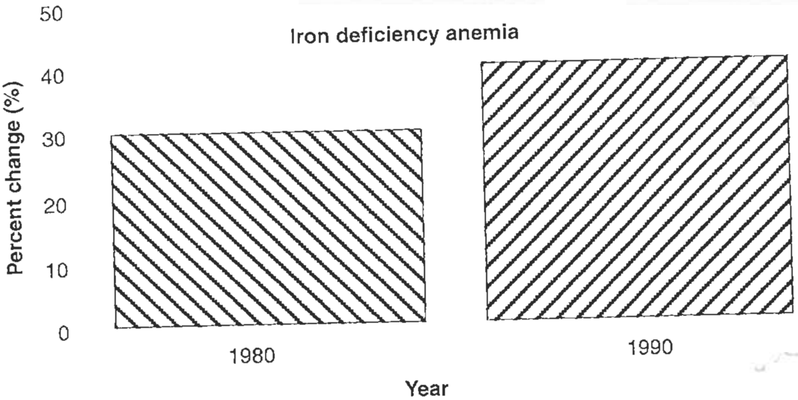

Figure 4 Global trends in the prevalence of iron deficiency anemia between 1980 and 1990 (values from Uvin, 1994).

people every day (see Fig. 4 for examples of global trends in iron deficiency anemia) (Combs, Jr. *et al.*, 1997). Agricultural systems must increase micronutrient outputs as a primary tool to eliminate micronutrient malnutrition (Combs *et al.*, 1996; Welch *et al.*, 1997). Finding sustainable solutions to this developing global nutrition crisis will not be possible without the cooperation of agriculture.

In the past, supplementation and fortification programs have treated the symptoms of micronutrient malnutrition rather than the underlying causes. While many of these interventions have been successful in the short term for the individuals reached by them, they have proved to be unsustainable and incapable of reaching all the people affected. Indeed, they are least likely to reach those most at risk, namely resource-poor women, infants, and children that live in remote areas either far from a clinic or those who do not have ready access to processed and fortified foods. In spite of these interventions, the problem continues to increase.

In developing countries, the rise in micronutrient deficiencies is linked to the shift in cultivation towards dominance by cereals. Pressure on a fixed land base to produce more food has driven a shift in production toward cereals. High cereal productivity, the result of extensive research, has ensured that cereal production is relatively profitable with a relatively low risk of failure through disease, drought, or post-harvest spoilage. Cereals are generally low in micronutrients, compared to many other food crops. Consequently, food systems dominated by cereals are low in micronutrients. Moreover, we do not see that this trend can be reversed while the global population growth rate remains high.

To address micronutrient deficiencies in the comprehensive way that the figures above demand, several approaches are needed simultaneously. The requisite agricultural research to correct these deficiencies will take some time to come on line even if funded in proportion to the magnitude of the problem. Therefore, all current interventions, where cost-effective, should be continued to treat as many people currently at risk as possible.

The development of new food systems to deliver the required nutrients sustainably will take much effort and research. Allocation of funds for agricultural research must take into account the balance of food items that can optimally satisfy nutrient and energy requirements. Research must be devoted to the yield-improvement of nutrient-rich crops (e.g., legumes) that may have declined in production as a consequence of their being out competed by improved cereal cultivars. Finally, attention must be given to increasing the micronutrient density of the major staple food crops in order to help redress the decline in mineral and vitamin intakes. Results outlined in this paper show it is possible to shift the nutrient balance of cereals, and diets dominated by cereals, in the direction of better balance. Therefore, even when socio-economic factors make it difficult to change the diet (Gopalan, 1999), the nutrient balance of cropping systems where cereals figure prominently can be improved. Our paper addresses this last question, as we consider it the most promising of the sustainable agricultural options that might be delivered in the shortest time.

Much of our experience has come from involvement in a feasibility study conducted within the last five years with colleagues at several CGIAR centers, including the International Center for Tropical Agriculture (CIAT), the International Center for Wheat and Maize Improvement (CIMMYT), and the International Rice Research Institute (IRRI). The philosophy of the approach developed for the study, significant results obtained, and the major challenges still to be met will be reviewed. The overall aim is to make clear the way that all agricultural scientists need to adjust their thinking in order to help meet these challenges in the early part of the new millennium.

II. THE NEED: THE GLOBAL PERSPECTIVE ON HUMAN NUTRITION

Underwood (1998) has presented a view from data of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) on the extent of micronutrient deficiencies in the human population, which shows how perceptions of these debilitating deficiencies within the nutrition community over the past few years have been extended to the economic consequences of them. More than 3 billion people are deficient in iron, 1.5 billion are deficient in iodine or live at risk, and some 250 million preschool children are deficient in vitamin A (a nutrient normally delivered in plant foods in the precursor form, β-carotene). While these international organizations do not have any figures on the extent of zinc deficiency because of the lack of a simple clinical screening procedure, specialists in zinc nutrition consider zinc and iron deficiencies to be of similar extent and distribution. These three elements and vitamin A are of first priority in primary health care. Other micronutrient deficiencies of growing concern to the human nutrition community include selenium deficiency and boron deficiency, though their roles in human nutrition are only emerging. Concern for the vitamins, α-tocopherol, ascorbate, cobalamin, and folate are increasing. Calcium, having some characteristics of a macronutrient and some of a micronutrient, is a significant concern in early childhood (i.e., rickets) and old age (i.e., osteoporosis).

The three micronutrients, iron, zinc, and vitamin A are all involved in brain development in the critical sense that clinical effects can be seen at levels of deficiency commonly found in practice. When present in the pregnant female, or for up to two years *postpartum,* permanent damage to brain development in the offspring is possible. Thereafter, further loss of cognitive ability is found, but this component of loss is reversible by better nutrition. These deficiencies compromise immune competence, greatly increasing sensitivity to infectious disease-causing organisms, thereby increasing morbidity and mortality rates among those populations affected. Deficiencies of these nutrients curtail worker productivity; moreover, they interact in their function to aggravate the morbidity. For example, deficiencies of vitamins A and C decrease absorption of iron. All together, micronutrient deficiencies greatly contribute to the degenerative cycle of poverty as they limit the capacity of parents to earn an adequate income that limits the level of nutrition they can provide to their children, in turn limiting their children's work and cognitive potential (WHO, 1996).

The WHO data indicate that these micronutrient deficiencies appear to be increasing in prevalence. At the same time, FAO data indicate that diet diversity is declining as population pressure impacts on patterns of land use. Under high population density, cereals are often the most productive, most reliable, and the most profitable crops. Their dominance of the landscape has increased (Welch and Graham, 1999). When cereals dominate the diet, they are inadequate nutritionally, especially in micronutrients (both trace elements and certain vitamins) and certain amino acids. This restriction in diet diversity appears the most likely reason for the emergence of micronutrient deficiencies over the last 15–20 years in such extent and severity. Cereals provide the most calories to humans and dominate the diets of most resource-poor people in the world. One significant consequence of this premise is that the situation is unlikely to improve in terms of diet diversity until population declines, which is unlikely for nearly 50 years, barring calamity. The only conclusion to be reached, is that a major effort must be made to improve the micronutrient content of cereals, and other staples. There are ways that this effort may be done by both agricultural and food-processing industries. In many developing countries, agricultural solutions are expected to be the dominant answer as infrastructural limitations and poverty will curtail the impact of food processing approaches.

A. The Speed of Change

The time it takes for change in the nutritional status of a population to emerge as clinical deficiency has recently been shown in the district of Chakaria, Bangladesh. A French medical team (Fischer *et al.*, 1998) reported severe rickets in children. The diagnosis was calcium deficiency in the diet rather than the usual cause, vitamin D deficiency. Surprisingly, there is no word in their local language for rickets, indicating recent etiology of this disease. Indeed, there are few in that population today over 25 displaying residual signs of rickets that normally afflicts children during growth spurts. The problem has become significant only in the last 20 years or so. An obvious cause has not emerged from the studies to date but a likely scenario is in the changing cropping systems as increasing population has dictated changes in land use. The people in the region describe themselves culturally as rice and pulse (grain legume) eaters, but today their fields are dominated by rice. Rice as eaten (milled and polished) has much lower calcium contents than the pulses, as the analyses of foods from a local market and household showed. While orthopedic surgery can help the existing children in some moderately wealthy families, this is not a sustainable or desirable long-term solution. Food systems must be changed to deliver the required nutrients, in this case, calcium, in adequate amounts. Better agronomic practices could increase rice yields so that some land can be spared for production of foods that help to balance the diet nutritionally. Additionally, rice cultivars vary in their grain calcium and high-calcium lines could be introduced to contribute to the supply. In the case of calcium, it can be easily calculated that high-calcium rice will only contribute perhaps 20% of the required shortfall.

In addition to the gross visible deformities of the calcium-rickets problem, all evidence suggests that several other micronutrient deficiencies (e.g., riboflavin, vitamin A, zinc, and iron) afflict most sectors of this population and all these nutrients must be part of a satisfactory, sustainable food-systems approach for this community. This study demonstrates how fast the nutritional status of a population can change, and the need to view agriculture as a source of all nutrients, not just calories.

B. The Paradox of Iron

One of the paradoxes of iron is that the earth's crust has an abundance of iron, but aerobic life on earth struggles to get enough because of the insoluble nature of iron in an oxidizing environment. All life forms together only require a mere one trillionth of the total in the earth's soils. On exposure to water and air during weathering of rocks, iron becomes oxidized and extremely insoluble; it is not leached out of soils into the oceans by rain, except catastrophically by erosion. The extreme insolubility and immobility of iron in aerobic environments ensures its presence in all soils providing universal stores of iron for soil microbes, plants, animals, and humans. Yet, its very insolubility in the presence of air denies ready availability to living aerobic organisms. Sophisticated biological mechanisms have evolved to allow either dissolution and absorption of iron from the environment or acquisition of iron by parasitism and predation upon other living organisms. Many higher plants (excluding cereals) rely on root-cell membrane-bound ferric reductases to acquire iron from soil solution in the form of the ferrous ion. In the case of the cereals that are low in these reductases, the chelates excreted by their roots are highly sophisticated phytometallophores that are absorbed intact by root cells via a specific ferric-phytometallophore transport system in the cell plasma membrane. These mechanisms allow plants to acquire iron that is otherwise tightly bound in soil, and this iron is then fed into the food chain.

Another paradox of iron is that it can be a highly toxic element when absorbed by aerobic organisms in excessive amounts because it can undergo a series of chemical reactions with oxygen, ultimately producing highly reactive and damaging oxygen free radicals (e.g., the hydroxyl free radical). Thus, iron uptake by all aerobic life is highly regulated to prevent excessive levels of iron from being accumulated in cells. For this reason, trying to make food crops absorb significantly more iron than that required to meet metabolic demands is difficult and must be done with care.

Iron deficiency occurs mostly in infants, children, and pre-menopausal (especially pregnant) women that are dependent on plant foods as their major source of iron. Monogastric animals, particularly young pigs, are also commonly deficient in iron if housed indoors during winter and fed feeds primarily composed of cereal grain. Grazing animals appear to pick up significant iron by soil ingestion. This iron is made available either by rumen microflora or by the acid conditions in the true stomach. Because of their iron absorption efficiency, cereals provide an important entry point for iron into the food chain, even though the concentrations of iron in the grain are quite low compared to meat-iron sources. The bioavailability of iron in plant foods is important as not all iron can be absorbed and utilized from the monogastric gut. Some iron is required by the gut microflora and fauna, and some is simply unavailable for absorption because it remains in forms that are insoluble.

C. Plant Foods as Sources of Micronutrients for Humans

Humans require the following trace elements and vitamins for growth and health: arsenic, boron, chromium, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, vanadium, and zinc; water-soluble vitamins ascorbic acid, biotin, cobalamin, folic acid, niacin, pantothenic acid, pyridoxine, riboflavin, thiamin; fat-soluble vitamins retinoic acid, calciferol, tocopherol, phylloquinone, and menoquinone. The bioavailability to humans of the fat-soluble vitamins requires adequate fat in the diet (Combs, 1998). Undoubtedly, more essential trace elements and vitamins will be added to the list in future.

Widespread deficiency of these nutrients in developing countries curtails economic development and stability. Food diversity is the traditional means of ensuring a balanced diet containing all these micronutrients, but this is becoming increasingly difficult as the global population continues to increase. In announcing the urgency of addressing dietary deficiencies in human populations, WHO/FAO (United Nations, 1992) called on governments to fund food-based solutions. Plant foods are able to supply all these micronutrients in adequate amounts with the exception of cobalamin, which comes mostly from animal products and bacterial contaminants on plant food.

III. NEW DIRECTIONS FOR WORLD AGRICULTURE

The path of development set down for modern agriculture to follow into the twenty-first century changed only a decade ago, but we need further change for the new millennium. The necessary changes have much to do with the impact of the "green revolution" and its perceived failings. Thus far, the changes in agricultural thinking have addressed only one of the major shortcomings of modern, technological agriculture, namely, the environmental concerns. Evidence, increasingly alarming, is showing us that in spite of our technology, modern agriculture is failing to deliver nutritious food and adequate nutrient output.

A. The Old Paradigm

Progress in agriculture in the last 100 years has been science-driven, producing an increasingly technological operation which has shown itself capable of achieving each lift in productivity needed to feed the world and even to provide more calories per person. The technology included new varieties, chemicals ranging from mineral fertilizers to pesticides to synthetic plant hormones, and machines to supplement and replace the labor force. This technological revolution in agriculture we call *the production paradigm* and it culminated in the "green revolution," a series of highly orchestrated, global strategies developed under the threat of starvation, to expand the global production of food, ensuring we could feed the increasing human family. This tremendous effort began in the 1960s and achieved adequacy in world food production in just two decades, an effort for which Dr. N. E. Borlaug received the Nobel Peace Prize in 1980.

B. The Current Paradigm

Even as this monumental international effort for food sufficiency got under way, Rachel Carson had published her famous book, *Silent Spring,* depicting the threat to our environment from the indiscriminate use of toxic chemicals, much of it in "modern" agriculture. Her lead gradually became a popular movement as more and more instances of the danger and damage were publicized. Not only was there concern for the environment in general, but there was concern that the existing orientation to agricultural production and "efficiency" was threatening the resource base of land, soil, air, and water through processes such as loss of soil fertility by erosion, acidification, salinization, and desertification. It was in the mid-1980s that the existing philosophy was largely overrun and a new paradigm had begun. The *sustainability paradigm* signifies that we must have high productivity while preserving or improving the resource base of agriculture and the environment. The "green revolution" failed initially to place enough emphasis on the sustainability of the increased productivity it set out to achieve.

C. A New Paradigm

No sooner had agricultural research become established within the new philosophy of sustainable agriculture—with remarkable global consensus (though there is still much to be done to realize our objective of sustainability)—than new concerns have arisen. In a way, like the concerns about the environment, these new concerns have been brewing for some time, but they have been brought into sharp focus by the data and statistics of the nutrition community and the WHO in the last few years. The food supply, while it has been sufficient (wars and the like excepted), is simply not nutritious enough (Section II). No previous disease or deficiency has ever affected over half of the total global population, in reality most of the women and children of the Third World, together with a surprisingly large number in developed countries. Some blame the "green revolution" in that the new highly productive cereals did not provide nutrient balance, but we believe it is more rational to attribute any blame to the population increase. As the basis of the effort to increase food production in poor countries, highly productive cereals have displaced other crops that are higher in iron. For example, in India where cereal production has increased more than four times in the two decades since 1970 (while the population has less than doubled), pulse production actually declined. To put an economic perspective on the magnitude of this problem, the World Bank has estimated that iron deficiency in India costs that country about 5% of its GNP annually, and 11% in Bangladesh. During the "green revolution's" push towards food security, little thought was given to nutritional value, and certainly almost none to the iron content of the new cereal varieties being bred, let alone to the iron content of the changing diets. To be fair, it must be said that the contemplation of mass starvation, inevitable without the "green revolution" effort, is far worse than the problems we must now tackle. The challenge now is to support a new paradigm for agriculture—an agriculture which aims not only for productivity and sustainability, but also, for balanced nutrition, or what we have called the *productive, sustainable, nutritious food systems paradigm.*

IV. AGRICULTURAL STRATEGIES FOR IMPROVING MICRONUTRIENT CONCENTRATIONS IN PLANT FOODS

There are a number of ways in which the micronutrient density of crop plants can be increased, and the necessary research to introduce these strategies successfully will require considerable laboratory support. Not only will agronomists and analysts need to understand the global situation and the complexities of a balanced human diet, but government policies will be needed to develop consumer awareness. Food industries need to be aware of the issues, whether they are major importers or exporters of food and food products.

A. Food Systems

Historically, nutritionists have formulated balanced diets by selecting from the foodstuffs available—a combination of food products that together deliver all known dietary requirements in reasonable proportions. We have seen above, however, that population pressure has changed the balance of foodstuffs produced. Sometimes this has been favorable to health (Gopalan, 1999), but it is now the likely cause of widespread and increasing micronutrient malnutrition in many countries. Given that these changes were driven by economics, food systems for the twenty-first century will need to consider diet diversity in an economic way rather than from the viewpoint of the dietary ideal. What is needed are nutritionists working with agriculturalists, economists, policy makers, and sociologists to consider food systems that are economically viable yet can deliver more micronutrients in an acceptable food supply. This will require consideration not only of the demand for calories and protein, but also their suitability in the cropping system and the labor demand and timing of any proposed new crop that might shift the diet in the desired direction. The economics of production, dietary balance, and consumer acceptability will be the final arbiters of change. Developing better diets in isolation from the economics and practicalities of the food systems is unlikely to succeed until population pressure is no longer the primary driver.

B. Fertilizers and Organic Amendments

Fertilizer technology and use are widely understood and appreciated in modern agriculture. It is a major vehicle for change in plant mineral content and food quality. The density of several micronutrients can be usefully enhanced by application of the appropriate mineral forms (Allaway, 1986; House and Welch, 1989): zinc, iodine, selenium, copper, and nickel. The effect of zinc fertilizer is shown in Table V, which also presents genotypic differences in ability to load zinc into grain. Although in this example the cultivar, Excalibur, is the best adapted to the zinc-deficient soil and has the highest yield with and without fertilizer added, it does not have as high concentration of zinc in grain as the new breeders' line, VL660. It is desirable to combine the traits of high yield (tolerance to the deficiency in the soil) and high nutrient density in grain (see Section V). Manganese can be increased only by late foliar applications to the generative tissues (Ascher, 1994).

However, because of its rapid oxidation in soil and because of its low mobility in phloem, soluble ferrous fertilizer is ineffective in increasing the iron concentration in plants, especially in the grain that develops months after application. Foliar applications are not much better. Chromium, boron, and vanadium are also ineffective fertilizers because of their low phloem mobility (Welch, 1986). All the vitamins of plant origin are synthesized *de novo* by the plant and are not a consideration as fertilizers. Thus, for many of the mineral nutrients of concern, fertilization is a useful strategy, while for iron and the vitamins, it is not (although adequate nutrition is a prerequisite for optimum vitamin biosynthesis in plants). Nitrogen fertilizer can increase vitamin content, but excessive application appears to be generally counterproductive (Salunkhe and Desai, 1988) and is to be avoided in any case for well-known agronomic and environmental reasons. Potassium

Table V

Zinc Concentrations (mg/kg, dry wt) In the Grain of Wheat Cultivars Grown on Zinc-Deficient Soil at Birchip, Victoria, With and Without Zinc Fertilizer Added to the Soil at Sowing

	Zinc concentration	
Cultivar	−Zn	+Zn
Declic	9.9	22.3
Songlen	10.8	27.3
Excalibur	10.8	22.3
VL660	13.7	29.3
LSD ($p = 0.05$)	3.4	

From R. D. Graham and J. Lewis, unpublished.

fertilizers often increase the concentration of vitamin C. Lime is used to increase the pH of acid soils and can enhance the calcium concentrations in plants, but its use is well-known to decrease the concentrations of the micronutrient cations. On the other hand, organic amendments, especially farmyard manures, increase the concentration of many nutrients and can be seen to enhance the nutritional value and nutrient balance of plant foods.

C. Varietal Selection and Plant Breeding

Exploiting the genetic variation in crop plants for micronutrient density is one of the most powerful tools we have to change the nutrient balance of a given diet on a large scale. Whereas conventional supplementation and fortification programs have been shown not to work well where the infrastructure is inadequate, delivery of nutrients in staple foods is certain to reach even the most disadvantaged. New varieties for farmers are the most widely and quickly adopted, and therefore, the most effective of modern agricultural technologies. This subject is discussed in detail in Sections V and VI of this review.

D. Molecular-Genetic Crop Transformation

Recent achievements in genetic engineering of new crop plants holds the promise of dramatic improvements in nutritional balance from fewer dietary components where the latter may be dictated by the agricultural consequences of excessive population pressure on food producing land. Genetic engineering involves first introducing foreign DNA into a crop species or artificially modifying its own DNA to achieve desired results. The process is such that often only a single cell is transformed in the desired way and it is then necessary to regenerate a whole plant from that cell by potentiating its whole complement of DNA through certain tissue culture techniques. An exciting recent example is the transformation of rice in Japan to have higher iron content in the endosperm of the grain (Goto *et al.*, 1999). In this work, the gene from soybean that controls the synthesis of phytoferritin, a large iron-containing protein, was inserted into the rice genome. This was accomplished using an endosperm promoter, the rice seed-storage protein glutelin promoter, *GluB-1*, that encodes the expression of phytoferritin more in the endosperm, where it can be most effective in human nutrition. On average, the iron concentration in the rice grains was doubled, but potentially this advantage could be even greater after milling, as up to half of normal iron in rice is in the outer layers and subject to loss in milling and polishing. Presumably, much of the phytoferritin would be retained after milling because some is located in the inner tissues (endosperm). While there is considerable debate about the bioavailability to humans of ferritin-iron from plant sources, and while the stability of such transformants is still a problem for this emerging technology, this success is a most encouraging pointer to the future. Indeed, several groups are attempting to transform other crops in the same way, and yet more are trying to transform food crops with hemoglobin-like molecules (see Section VII).

E. Farm Management

Crop rotation is an important tool in food production as well as soil management. Rotation of cereals and grain legumes allows for better management of difficult weeds and the input of nitrogen fixed by the legume is important to the overall nitrogen balance and cost. At the same time, these two crops provide some nutritional balance to the diet. For example, while cereals are low in iron, manganese, copper, cobalt, calcium and magnesium, grain legumes are relatively high. While grain legumes are very low in sulfur amino acids, the cereals are relatively better. In South Asia, cereal production has increased four times faster than that of grain legumes in the last 30 years (FAO production statistics). More productive grain legumes could redress this imbalance. Both these groups of staples are low in many important vitamins that can be supplied by vegetables and fruits (Combs, 1995), but the introduction of more balanced cropping systems in near-subsistence circumstances must occur in a suitable economic environment if they are to survive and have an impact. This involves a complex balance of factors that requires participation from several disciplines.

Tillage systems are changing, but the impact on plant composition is not currently considered, especially in respect to nutritional quality. We know that the shift towards minimum tillage tends to decrease nutrient density as deficiencies are more common, especially of nitrogen and phosphorus, but also of micronutrients. The long-term effects of such changes in cropping systems need to be fully understood.

V. THE CASE FOR PLANT BREEDING

The breeding of crop plants for nutritional quality has been strongly questioned by many plant breeders because the history of past attempts is not encouraging. The primary nutritional objective in the last 50 years has been to improve the protein content and quality of staple crops. This has been quite difficult and has achieved only limited success. Nutritionists also question this strategy as, traditionally, they have advocated attaining nutritional balance by dietary diversity. The collective wisdom has therefore been that in a world of expanding population, plant breeders of staples should focus on delivering calories, leaving nutritionists to deal with the need for dietary diversity to achieve balanced nutrition.

The issues of this debate are being raised again in the International Agricultural Research Centers (IARCs) while means of dealing with the extent and severity of micronutrient deficiencies in human populations are considered from every angle. As the IARCs debate the value of breeding for micronutrient-dense cultivars of staple crops, it is worth remembering that in order to make a sound economic assessment of the potential contribution of breeding to controlling micronutrient malnutrition, more *biological* information is needed than currently exists. The feasibility and costs of breeding can only then be assessed in relation to the magnitude of benefits to malnourished consumers. These benefits must be assessed from feeding trials (bioavailability studies) on the best germplasm identifiable. This section is intended to be a contribution to the debate, but more, it sets out the deficiencies in our knowledge on which we must base an informed opinion.

It is something of a circular argument that an economic analysis should be done to justify research expenditure in this area but some research expenditure is needed in advance to generate the data on which a realistic economic analysis can be based. We argue that efforts to identify and collect the essential biological information on germplasm resources, heritabilities, efficacy of selection criteria or molecular markers, as well as bioavailability of the extra micronutrient contained in the best germplasm found is justified ahead of a wide-ranging economic analysis.

The fundamental assumption in proposing a plant breeding contribution to overcoming micronutrient malnutrition is that nutrient density traits must be delivered in cultivars of the highest yield. In order to have maximum impact, top yielding lines are needed to convince local farmers to grow them when the target consumer is in no position to pay a higher price for quality. To do this, a major increase in breeding costs will be necessary in order to maintain progress in yield and quality concurrently, and the results of exploratory research will need to justify that increase to the donors supporting the major breeding programs in the international centers.

For a plant breeding approach, as with fertilizer use, laboratory support is essential. Selecting among existing varieties is the simplest approach. Results of a study at CIMMYT show that of the wheat varieties released by that organization over 40 years of breeding, the best were about 20% higher in iron and zinc concentration in grain than the lowest (Ortiz-Monasterio, 1997, Graham *et al.*, 1999). A 20% difference is likely to be important to deficient consumers of this staple, provided they are capable of absorbing the iron. Surprisingly, the overall iron concentrations in grain were not depressed by the increased yield achieved over 40 years of breeding. The same seems to be true for *Phaseolus* beans. Over the whole process of domestication and modern plant breeding, despite a huge increase in yield potential, the mean concentrations and the range across 1,100 varieties and 200 wild types of beans were about the same for both iron and zinc (data of S. Beebe in Graham *et al.*, 1999).

Predictably, the potential for nutritional enhancement by deliberate selection within a breeding program is much greater than by selection within currently available varieties (Gerloff and Gabelman, 1983; Graham and Welch, 1996). We have found four- or fivefold variations between the lowest and highest micronutrient cation concentrations in the grain of several hundreds of accessions from the germplasm banks of the major cereals (Graham *et al.*, 1999); the highest concentrations are about twice those of popular modern cultivars. For β-carotene in maize and cassava, the range is much greater than that for iron (Brunson and Quackenbush, 1962; Iglesias *et al.*, 1997). In all crops studied, it is possible to combine the high-density trait with high yield, unlike protein content and yield that are negatively correlated. The micronutrient traits are stable across environments and the genetic control is relatively simple. For example, high iron density in rice is linked with aromaticity, making selection easier in early generations (Graham *et al.*, 1997).

Screening for micronutrient cation density is easy using the inductively coupled plasma optical emission spectrometer (ICP-OES) after nitric-perchloric digestion. The cost is considered high for breeding work and ways of lowering the cost for dedicated servicing of plant breeding programs is needed. The alternative is to use the ICP-OES capability to help develop molecular markers that are expected to cost around $ U.S. 2.00 per sample tested, somewhat less than the ICP-OES laboratory. Ultimately, total nutrient concentration is not the objective, but utilizable nutrient in the human gut. The bioavailability of the nutrient in high-density types must be demonstrated, and this requires special feeding trials by human nutritionists. These are so expensive that they are only possible for advanced lines scheduled for release.

The micronutrient density of seeds is important not only for human nutrition, but also for animal production and the nutrition of the seedling in the next generation (section VI). A vigorous crop is established by seeds with a high density of nutrients, including micronutrients (Welch, 1986; Crosbie *et al.*, 1993; Rengel and Graham, 1995; Mousavi-Nik *et al.*, 1997), that confer better resistance to stress and disease, leading eventually to higher yield. These effects are most pronounced in micronutrient-deficient soils (Weir and Hudson, 1966; Gurley and Giddens, 1969; Longnecker *et al.*, 1988; Yilmaz *et al.*, 1997).

The prospects for improving the provitamin A carotenoid density of staple plant foods are very good indeed. Yellow types are well-known in all the usual white starchy plant foods except rice, although the genetics are complex and somewhat obscure. Rapid breeding progress should be possible since genetic gain in β-carotene content can be visually estimated with accuracy (Simon, 1992). Moreover, the carotenoids tend to be endosperm-stored, and therefore less subject to the milling losses seen for mineral content in cereal grains. It is necessary, therefore, only to characterize the parental material to establish that the sources of pigmentation do have high provitamin A activity in humans.

VI. GERMPLASM RESOURCES, GENETICS, G*E, HIGH YIELD, SEEDLING VIGOR

A. Germplasm Resources

Germplasm resources can be exploited to improve the nutrient output of food systems in several ways. The simplest is by designing new cropping systems with better nutrient output, for example, by re-introducing micronutrient-dense pulse species into cereal-dominated production systems. However, in many places the trend has been exactly the reverse, driven by the forces of population pressure and economics, and so cannot immediately be sustainably reversed. There is an urgent need to reduce the risk to farmers associated with pulse production and to enhance the yield and reliability of most pulses, while maintaining or further enhancing their nutrient density. Through increased yield, the caloric output of the pulse phase of the rotation contributes both to the overarching need for calories and protein, and to adequate returns for the farmer. Only then will these cereal-legume rotational systems be productive, sustainable and nutritionally enhanced.

Within any food system, selecting micronutrient-dense lines among existing varieties is the first and easiest approach. Micronutrient-rich cultivars of wheat had 20% more micronutrients than other high-yielding releases (Ortiz-Monasterio in Graham *et al.*, 1999). This difference is likely to be nutritionally important to deficient consumers of wheat products, provided % bioavailability is at least the same. Implementation of this simple strategy needs the establishment and acceptance of the importance of nutritional quality as a breeding objective by the breeders concerned and/or by agronomists who influence the choice of crops and cultivars in farming systems. The adoption of nutritional objectives by the breeding program is important in choosing lines for registration and release. Breeders often have several sibs to choose from on the basis of small differences in expression of many traits. In making the decision, differences in nutrient output could be a priority when the breeders and their stakeholders rate it highly enough.

B. Exploiting Genetic Variation in Nutrient Composition

Much greater than the passive-empirical approach above is the potential for nutritional enhancement within the breeding program by deliberate use of nutrient-dense parents followed by selection in segregating populations (Gerloff and Gabelman, 1983; Graham and Welch, 1996). The data in Fig. 5 are typical of the genetic variation in seed nutrient concentrations among cultivars and breeders' lines in field trials. In this field trial, entries were mostly released cultivars or ad-

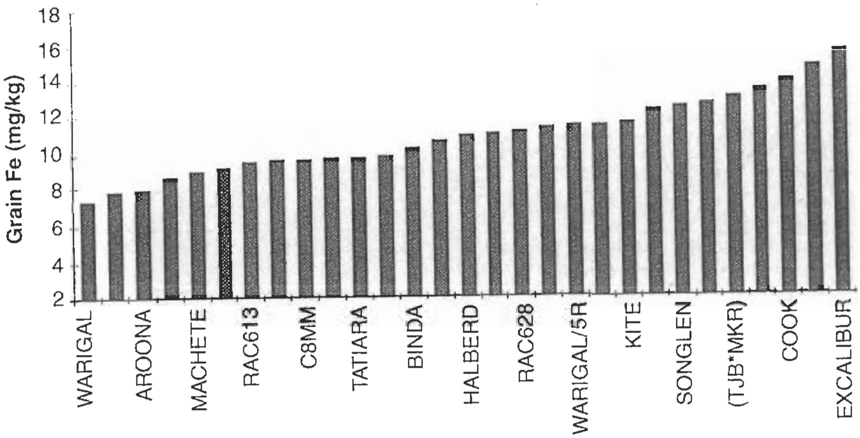

Figure 5 Iron concentrations in wheat grain of 30 cultivars grown together in a field at Lameroo, South Australia in 1988. Means of five replicate plots. Note that the best is about 2.5 times that of the lowest density cultivar.

vanced lines from local wheat breeders. The best varieties were about 2.5 times higher in iron concentration than the poorest, and the most zinc-dense lines were among the more iron-dense lines. Since the entries in this trial were not genetically as diverse as one would find in a germplasm bank, the range of concentrations is not as great as that described below. Moreover, the mean concentration is relatively low as soil type and other environmental factors do contribute to the expression of this phenotype, therefore, the range of values in this high pH sandy soil is lower than that seen in better soils. However, the best varieties tend to be the best in all environments.

Since environmental factors affect seed nutrient concentration, the only true and useful comparison of the composition in seed of many genotypes comes from growing them together in the same field in the same season and analyzing the seed produced rather than the seed sown, as the latter may have come from diverse environments or seasons (see section VI-G). This technique was followed throughout the study described below. Within experimental error, all differences could be ascribed, *prima facie*, to the genotype effect, although effects of unusual genotype *x* environment interactions cannot be ruled out.

C. Results of the CGIAR Micronutrient Project

Given that such genetic variation was known to exist and has been repeatedly demonstrated, we might ask the question, what is the full range of variation in a major germplasm bank for the traits of interest? The CGIAR was funded by the Danish International Development Agency to explore the potential in its germplasm banks of the major staple crops for micronutrient density. As a major report was published recently (Graham *et al.*, 1999), a summary of our findings and interpretations is presented here. (Besides the authors named, Drs. G. Gregorio, W. Roca, H. Cabellos, and M. Banziger have contributed to this effort.)

Wheat: From a survey of CIMMYT wheat germplasm, Dr. Ortiz-Monasterio found four or fivefold variation between the lowest and highest iron and zinc concentrations in the grain among several hundreds of accessions. The highest concentrations were about twice those of popular modern cultivars, so that a factor of two is the likely potential for improvement over the currently grown varieties. However, Ortiz-Monasterio (1998) has found iron and zinc density in wild relatives of modern bread wheats to be even greater (100 and 140 mg/kg respectively), with up to 50% more again. Since the seeds of these wild progenitors are often small, it remains to be shown whether their iron and zinc density will still be fully expressed when the trait is introgressed into high-yielding cultivars.

Generally, what has been found for iron and zinc, has also been shown for other minerals in grain. Moreover, there is a positive correlation of iron and zinc concentrations in grain. This significant, but not perfect, "linkage drag" means that screening for iron alone is likely to improve the concentrations of several minerals. This correlation is also significant for iron/zinc and calcium, three cations of special importance to human nutrition. Lines especially high in both iron and zinc have been found, and moreover, among high-yielding lines, it has been demonstrated that the high-density traits can be combined with high yield. With micronutrient density traits, there appears to be no (or very little) negative correlation with yield. This is a most important point in view of our strategy of getting these benefits to all consumers via varieties that farmers will want to grow. The fact that we may be able to raise the concentrations of all or several minerals together, and especially the more critical ones, will benefit consumers in terms of nutritional balance as well.

Rice: The findings in the rice project are particularly encouraging. Iron density in rice varied from 7–24 mg kg^{-1} (all concentrations reported are on a dry weight basis) and zinc density from 16–58 mg kg^{-1}. A benchmark was established in that nearly all the widely grown "green revolution" varieties were similar, about 12 and 22 mg kg^{-1} for iron and zinc, respectively. The best lines discovered in the survey of the germplasm collection were therefore twice as high in iron and 1.5 times as high in zinc as the most widely grown varieties today. High iron and to a lesser extent, high zinc concentration, were subsequently shown to be linked to the trait of aromaticity. Most aromatic rices such as jasmine and basmati types are high in both iron and zinc, and as before, generally in most minerals (Senadhira and Graham, 1999; Graham *et al.*, 1997, 1999). Recently, four loci contributing to iron density in rice grain has been identified, two of them loosely linked to loci from aroma (G. Gregorio, personal communication). As in other crops, these micronutrient density traits have been combined with high yield.

In addition to genetic variation in nutrient density in rice grains, there are genotypic differences in the relative loss of iron and zinc in the milling process. It is because iron is inherently low in rice and because milling removes half or more of that, rice is the poorest in iron of all the cereals.

Maize: A cereal with enormous yield potential, maize offers equally great potential nutritionally, although this is not generally accepted. Generally, maize kernels have higher iron and zinc concentrations than grains of the other high yielding cereals. The project's leader, Dr. Banziger has shown that the potential to increase these concentrations by further deliberate selection within high-yielding germplasm appears to be less than in wheat or rice. However, multiple aleurone layer (MAL) types are known and have been shown to have higher concentrations of all minerals than normal single layer maize (Welch, unpublished). Modern maize also has much variation for protein and the modern high-lysine types offer better amino acid balance in this limiting nutrient. Tissue culture selection (Phillips and McLure, 1985) has made available high methionine germplasm that further enhances the quality of the protein and potentially the absorption of iron and zinc in the gut (Section VII). Most importantly, in maize there are high β-carotene types (yellow maize) that are very high yielding and available in combination with high-lysine and the other nutritional traits. Bearing in mind the synergistic interactions among nutrients, this represents the best-balanced nutritional package available among the most productive staple crops. While it is obviously deficient in vitamin C and tryptophan, a nutritionally enhanced maize has the potential to help balance a poor diet that is based on very few components.

Beans: Dr. Beebe has shown considerable genetic variation in mineral concentrations among both wild beans and modern cultivars but domestication has not changed the mean concentrations of iron and zinc in the seeds, nor the range. This is most surprising, given the marked increase in yield between wild and modern beans; obviously, the loading of iron and zinc into the bean seed has increased in proportion to the yield over the time of domestication. The concentration of zinc in beans is one of the highest among vegetable sources, and is nearly equal to dairy products (Pennington and Young, 1990). Unlike wheat and rice, the concentrations of iron are generally even higher than those of zinc, but like the cereals, iron and zinc are positively correlated. However, unlike the cereals, there is no correlation with calcium. The concentrations of iron at the high-end (100 mg kg^{-1}) are much higher than in the cereals, although relatively, the increase that is possible through deliberate selection is less. Again, like wheat and rice, the high-density traits are fairly stable across environments, although, as in all crops, the environmental effect is also highly significant.

Beans are high in phytate and tannins, both considered antinutrients that inhibit the absorption of dietary iron and zinc from the gut. However, in the most comprehensive studies done to date, the bioavailability of the iron and zinc to rats and to humans is not dependent on the concentrations of either antinutrient, even though the amounts present varied over a wide range (see Section VI for details). Genetic variation for these antinutrients exists but its exploitation depends on further bioavailability data to justify the effort and to define whether to increase or to decrease these constituents. As discussed in Section VI, there are nutritional arguments for not decreasing their concentrations. On the other hand, promoters of absorption, such as the sulfur-containing amino acids, also vary with genotype and have heritabilities that make breeding practical, if needed. Since these amino acids are generally low in beans, as in many pulses, such a breeding effort would appear worthwhile, though the potential for increasing them may be only of the order of 20–30% of the current mean concentrations.

Cassava: Like maize, cassava has much more potential nutritionally than is generally accepted. Its poor reputation is probably because the root has low protein concentration, and dates from the 1960s when protein was considered the primary limiting factor for nutrition. Cassava leaves and roots generally contain cyanogenic glycosides and so require careful processing to eliminate the cyanide before consumption. The toxin is apparently useful in protecting the crop from insects, wild pigs, and other animals, so the crop can be stored in the ground as a drought reserve. However, sweet types exist that require less in the way of special preparation to remove the cyanide. The CIAT team, Drs. Iglesias, Roca, Bellotti, and Cabellos, have advocated the use of the leaves as a green vegetable because some Brazilian populations depend on leaves from this crop for additional nourishment. The study of the leaves strongly indicates considerable nutritional value, since they are high in protein, iron, zinc, and other minerals, as well as vitamins A, B, and C. They are, therefore, a useful supplement to the roots that provide lots of energy but are low in protein. Even so, studies have shown that there is considerable genetic variation for root protein, iron, zinc, calcium, β-carotene, and vitamin C. At the high end of the range, these concentrations can make an important contribution to nutrition. For example, the concentration of vitamin C in the roots can be as high as 40 mg kg^{-1} (fresh weight basis), and the iron can be as high as 10 mg kg^{-1}, higher than in most milled rice. In the Brazilian collection there are many accessions of cassava with yellow and orange-colored storage roots. For cassava roots, β-carotene varies from nil to 2.5 mg kg^{-1} of fresh root, sufficient to supply the recommended intake of a child from a portion the size of a small potato (Iglesias *et al.*, 1997). They have shown that the genetic control of β-carotene, in a cross of high × low pigmented types, is by two genes with epistatic effect controlling respectively transport and loading of precursors. Genetic variation has also been shown in the extent of losses in nutritional value resulting from cooking and processing, a potentially fruitful area of research in food systems for health.

Carotenoids: The prospects for improving the provitamin A carotenoid density of staple plant foods are very good. Yellow types are well-known in all the usual white starchy plant foods, but less known in rice. Although the genetics are complex and somewhat obscure, rapid breeding progress is nevertheless possible since genetic gain in carotene content can be visually estimated with accuracy (Simon, 1992). Recently, carotenoids other than β-carotene have been shown to be present in the eye where they function in preventing age-related macular degeneration (Khachik *et al.,* 1999). Such carotenoids are contained in many vegetables and fruits, as well as, pasta and bread wheats (Rosser *et al.,* 1999). Strong carotenoid pigmentation was common in older bread wheat varieties, and yellow bread is still common in China where it is prized for its aroma and taste. In this century, wheat breeding has been focused on wheats producing white flour and driven by market demand. The traits for high grain carotenoid content have been eliminated. By re-educating consumers, these types could be brought back into breeding programs in all countries, but the benefits would be greater for resource-poor consumers. The importance of carotenes to iron deficiency anemia is indicated by the recent work of Garcia-Casal *et al.* (1998) who showed that both vitamin A and β-carotene increased the gut absorption of iron from fortified cereal-based meals (see Section VII). It remains to be seen whether other carotenoids can have the same effect. Carotenoids abound in cereal germplasm, and even β-carotene can be increased in wheat through breeding. It is already high in yellow maize and has eliminated vitamin A deficiency in pigs housed in winter (Brunson and Quackenbush, 1962). The significance of carotenoids in cereals is the high intake of these staples and the chemical diversity of carotenoids present, so giving a spectrum of potential for quenching damaging free-radical reactions within cells.

D. Genotype Environment Interactions

Expression of the micronutrient-density traits has been tested over a wide range of environments, and although the environmental effect itself is strong, the genotype effect is consistent across environments (implying the G*E interaction is not serious), sufficient to encourage a breeding effort. Environmental factors considered by one or more of the crop programs include acid soils, alkaline soils, saline soils, acid-sulfate soils, iron-deficient soils, time of planting, field site, season, nitrogen fertilization, phosphorus fertilization, potassium status, elevation (cold tolerance), and drought stress.

E. Genetics of Nutritional Traits

The first genetic study of a micronutrient efficiency factor was conducted by Weiss (1943) on iron efficiency in soybeans, in which it was shown that efficiency was due to a single, major, dominant gene controlling the reducing power of the root membrane surface. Since Weiss' pioneering study, several minor additive genes have been discovered to contribute to iron efficiency in this crop (Fehr, 1982). These are of practical significance in improving tolerance to iron-deficient soils as all successful cultivars carry the efficiency alleles at the major locus. A major and several minor genes are likely to be the case with other micronutrients. In his review of genetic control of plant nutrient traits, Epstein (1972) noted apparently simple genetic control of boron efficiency in tomato and celery, iron efficiency in maize and tomato, and magnesium efficiency in celery. Subsequently, another iron efficiency trait in tomato has been shown to be due to a major gene, coding for a ferrous-iron transporting non-protein amino acid, nicotianamine (Ripperger and Schreiber, 1982). This gene is expressed in the shoot and appears to facilitate iron transport in the phloem and so may play an important role in transport into fruits and seeds. Thus, two suites of genes appear to be important to loading micronutrients into seeds: those involved in uptake from soil, known as micronutrient efficiency traits (Graham, 1984), and those involved in transport within the plant and to the seed, nutrient transport and loading traits. Little is known of the genetics of transport and loading of micronutrients, other than the pioneering work on tomato mentioned above. Nicotianamine has since been detected in a wide range of higher plant species and may be a universal ferrous-iron ligand facilitating both intercellular and long-distance transport in the phloem. Indeed, it is likely to be able to facilitate the movement of all the micronutrient cations (Welch, 1995).

The genetic control of iron density in rice appears to be relatively simple. High iron and zinc density in rice is linked with aromaticity, making selection easy in early generations by smell (Graham *et al.,* 1997). Iron and zinc-dense rice lines have been selected in the breeding program at the IRRI that are high in yield, aroma, and cooking quality. They are superior in other minerals as well, and have proved to have high bioavailability to rats (currently subject to long-term human dietary trials in the Philippines).

Copper efficiency in rye appears to be a dominant trait controlled at a single locus on the long arm of chromosome 5R (Graham, 1984). Of several translocations to wheat, the 5RL/4A translocation appears to be the most satisfactory agronomic type and has been successfully incorporated into adapted cultivars for South Australia (Graham *et al.,* 1987). Work with rye addition and translocation lines has shown that copper efficiency is not linked to zinc efficiency, nor either of them to manganese efficiency. Thus, independent and relatively specific genes are involved, and root system geometry or size does not appear to be critical. For example, although wheat and triticale root systems are similar (Graham *et al.,* 1981) and the triticales do not inherit the fine and extensive root system of rye, yet they do inherit all three micronutrient efficiency traits from rye. Manganese efficiency is located on 2R, a conclusion supported by the poor performance on manganese-deficient soils of armadillo-type triticales lacking 2R. Manganese efficiency in barley also appears to be simply inherited (Graham, 1984; McCarthy *et al.,* 1988). A high percentage of wheat and barley cultivars currently have exceptional sensitivity to manganese deficiency, the genetic basis of which is unclear in wheat but in barley, many of them have a parent introduced from Alexandria, Egypt, in their pedigree.

Less is known of the genetics of zinc efficiency (Graham *et al.*, 1992). The study of addition lines of rye (Graham, 1984) suggests several loci on as many different chromosomes are involved in zinc efficiency in rye; likewise, a few genes are involved in zinc efficiency in rice. The largest single screening exercise was 3703 lines of paddy rice (Ponnamperuma, 1976; IRRI, 1979) where 388 lines were judged to be tolerant. Following diallel analysis, a recent report suggested that the genetic effects responsible for the zinc efficiency trait in rice are mostly additive, and to a lesser extent dominant (Majumder *et al.*, 1990). Soybean varieties differ in their response to zinc fertilizer (Rao *et al.*, 1977; Rose *et al.*, 1981; Saxena and Chandel, 1992). Such a result is suggested to be a consequence of differential efficiency of zinc absorption; the distribution of F3 lines from the cross between zinc-efficient and zinc-inefficient genotypes (330 F3 lines tested) suggested that only a few genes control the zinc efficiency trait (Hartwig *et al.*, 1991). Recently, a recombinant inbred population of *Phaseolus* beans and molecular-genetic analysis of QTLs were used to map several loci controlling zinc concentration in the bean seeds (Beebe, personal communication).

The various mechanisms of zinc efficiency are likely to be additive as suggested by Majumder *et al.* (1990), putting great emphasis in a breeding program on step-wise pyramiding of genetic information. Combining traits for several micronutrient efficiencies into one locally adapted crop cultivar has been facilitated by the availability of rapid methods of producing doubled haploid populations that can be immediately phenotyped by conventional bioassays. Using the best local germplasm, cultivars with improved zinc efficiency may be expedited without severely disrupting the broad adaptation already achieved. A recent Australian study has indicated that it is possible to pyramid zinc efficiency genes in bread wheat to produce much more efficient types than currently exist in released cultivars (Grewal *et al.*, 1997).

F. Plant Breeding for Nutritional Balance

Nutritionists stress that balancing diet is a complex issue. It not only depends on the diversity in the diet, but on what foods are eaten together as one influences the nutrients extracted from the other; for example, vitamin C from fruit can increase the absorption of iron from cereals. The food processing method also affects the outcome.

However, the CGIAR mission is empowering the poor through assisting in appropriate change to their agriculture. One of the poorest countries, Bangladesh, produces only 20% of the vegetables it requires by FAO standards. In all probability, this production is unevenly distributed to the wealthy, and millions of resource-poor citizens of Bangladesh eat virtually only cereal. This group is the target group, and any genuine improvement in the micronutrient concentration of their staple will fully benefit them. While this may be the simplest scenario, consider people in progressively higher socio-economic strata. Their diet will be diversified accordingly, they will eat less staple and benefit proportionately less, but they will also be less at risk.

This strategy for maximum effect through plant breeding should not, unwittingly, be projected as delivering a balanced diet, even though a cultivar might be greatly improved with respect to the most commonly deficient micronutrients, iron, zinc, and pro-vitamin A. Improved intakes of target nutrients would be expected to have a major effect in improving cognitive and physical capacity of the generation born after the new cultivar goes into wide production. Additional increments in health and welfare, through the supply of other less-limiting nutrients will depend on improved work capacity and income, leading to better diversity of diet as this new generation grows up. Thus, the primary benefit will be breaking the cycle of poverty due to the perinatal effects of these major deficiencies on the mother and child.

Selenium is an essential element whose roles are only emerging at this time but three aspects of it suggest that it should be included in the initial screening work: it is intimately involved in the metabolism of iodine which cannot function without it, and deficiency of it is exceptionally widespread. Thirdly, it can be analyzed in grain concurrently with iodine by ICP-OES or ICP-MS. In a recent paper, Combs (1995) recognized bread as a useful source of selenium, while another source noted purple wheats as rich in selenium. There would appear to be good opportunities for finding useful genetic variation in selenium accumulation in wheat grain. Finally, the link of low-selenium intake and cancers of almost every kind is now stronger (Clark *et al.*, 1996), therefore, work on selenium will attract funding from wealthy nations.

G. Seed Nutrient Content

The micronutrient density of seeds is important not only for human nutrition, but also for the nutrition of the seedling in the next generation. A more vigorous crop is established by seeds with a high density of nutrients, including micronutrients (Welch, 1986; Rengel and Graham, 1995; Mousavi-Nik *et al.*, 1997), that confer better resistance to stress and disease, leading eventually to higher yield. These effects are most pronounced in micronutrient-deficient soils (Weir and Hudson, 1966; Yilmaz *et al.*, 1997). Thus, micronutrient-dense seeds are desirable both for the farmer and the consumer, a "win-win" situation.

The seed is a store of nutrients that provides for the growth of the enclosed embryo to create the next generation. The embryo must draw on these nutrients to develop the leaves that can take over the role of providing energy to the seedling and to develop the roots that must ultimately deliver the mineral nutrients needed for further growth. It is necessary, therefore, that the seed contain enough nutrients, including micronutrients, to sustain root growth until there is enough root absorbing surface to supply all the young plant's needs. This is not a simple matter. The seed must have a bigger store of a particular nutrient when the soil is low in that nutrient, because it will take longer to create a root system of sufficient size to supply the plant at the pace required for maximal growth rate. Paradoxically but not surprisingly, it is under conditions of low nutrient availability in soil that the mother plant has most difficulty in storing adequate nutrient in the seed. This is a serious problem in agriculture such as in subsistence farming systems where farmers keep their own seed. These seeds are likely to be low in the nutrient that is also low in the soil, thereby compounding the problem of poor seedling vigor. Poor vigor, in turn, leads to poor establishment, more severe competition from weeds that are better adapted than the crop, more susceptibility to diseases, and ultimately lower grain yield. The costs in terms of grain yield from seeds of low nutrient content have been demonstrated for zinc in wheat (Marcar *et al.*, 1986; Rengel and Graham, 1995), manganese in barley (Longnecker *et al.*, 1991; Ascher, 1994) and lupin (Crosbie *et al.*, 1993), molybdenum in maize (Weir and Hudson, 1966), and phosphorus in lupin (Thompson *et al.*, 1990). Many more examples have been demonstrated under controlled experimental conditions, and it is likely to be a general phenomenon. However, the case of zinc is of greatest interest here. Zinc is widely deficient in soils, crops, animals, and humans, indeed the whole food chain. It follows, therefore, that breeding for zinc-dense seeds will benefit both producer and consumer. The agricultural imperative and the health imperative coincide. This makes the case for breeding nutrient-dense seed compelling.

Seedlings under stress from waterlogging, high pH, defoliation, or herbicide damage often first show symptoms of iron deficiency, indicating that the in-built iron scavenging mechanisms (Welch, 1995) have been compromised. It can be deduced from this that adequate stores of iron in seeds are equally important as for the other nutrients above, though this is more difficult to demonstrate experimentally because of the inefficiency of iron fertilizers needed for the control treatment.

There are further implications in the seed-nutrient effect. In breeding programs, it has been customary to grow breeding lines together in the same field trial that have been collected from various places. This is true whether the field trial is advanced breeders' lines, potential parents for the breeding program, or segregating materials from within the breeding program. For example, parent materials might be gathered from around the world or around the country to identify types adapted to a particular type of soil that is among other things, deficient in zinc. Such a variety trial will be confounded because the seed of the entries will vary in zinc according to their source and not solely to their genotype. Seed zinc content is controlled additively by both genotype and the environment of the mother plant. Thus, a line that has high seed zinc (because it was grown in a high-zinc soil) will have an advantage in the trial regardless of whether it has the genes for high zinc density or not. In the same way, a nutrient-dense genotype may be disadvantaged in this trial if it came from a zinc-deficient area. The implications of this are important for breeding in micronutrient-deficient areas and are equally so for breeding for high nutrient density. It is necessary to grow all seed together at the one site for at least one generation to level the playing field before looking for high-density phenotypes. Breeding programs in South Australia have recognized these constraints and adapted methods to deal with them. Of course, as we study the genetics of these traits, and map the genes and determine molecular markers for them, it will be possible to characterize the genotype directly, avoiding these confounding effects on the phenotype, and greatly speeding up the breeding process.

VII. THE IMPORTANCE OF BIOAVAILABILITY

Crop and soil scientists are fully cognizant of the importance of the "available" nutrient status of soils in determining fertilizer recommendations for optimizing crop productivity. They understand that the total concentration of a nutrient in a soil does not reflect the plant-available nutrient supply within the soil's rooting zone. This is especially true for micronutrient metals such as iron, zinc, copper, and manganese which can be present in soil pools in forms that are unavailable for absorption by plant roots (Marschner, 1995; Welch and House, 1984). This concept of nutrient availability also holds for micronutrients in plant foods eaten by people consuming varied diets containing a myriad of other food ingredients. The total amount of a micronutrient in a plant food does not represent the actual micronutrient content of the food that is utilizable by the consumer. This quantity (i.e., the bioavailable amount) must be determined independently using methodologies especially developed for such purposes. In human nutrition terms, bioavailability is commonly defined as the amount of a nutrient in a meal that is **absorbable** and **utilizable** by the person eating the meal (Van Campen and Glahn, 1999). There is an immense body of research concerning micronutrient bioavailability in plant foods that cannot be covered in the brief review of micronutrient bioavailability presented here. For more detailed information concerning this topic the reader is referred to the following references: (Benito and Miller, 1998; Fairweather-Tait and Hurrell, 1996; House, 1999; Hunt, 1996; Hurrell, 1997; Matzke, 1998; Rao, 1994; Van Campen and Glahn, 1999; Wienk *et al.*, 1999; World Health Organization, 1996).

A. The Complexities of Bioavailability

Fig. 6 along with the text box below depicts the dynamic factors and their interactions that affect the amount of a micronutrient bioavailable to a person eating a meal containing plant food. Micronutrients can occur in various chemical forms of differing proportions in plant foods and their amounts vary depending on numerous factors including the growth environment, plant species, genotype, and cultural methods used to grow the plant. These forms have characteristically different solubilities and reactivities with other plant constituents and other meal components. There are multiple interactions occurring between micronutrients in plant foods and other plant substances once the food is consumed, such as with other interacting nutrients and chemical substances that can either inhibit (i.e., antinutrients) or enhance (i.e., promoters that can increase absorption and/or utilization) micronutrient bioavailability. Additionally, many other interacting factors, both genetic and environmental, affect micronutrient bioavailability to the consumer, such as food processing methods, meal preparation techniques, and an individual's personal characteristics (e.g., sex, age, genetic predisposition, ethnic background, economic status, physiological state, nutritional, and disease status). Thus, determining micronutrient bioavailability in plant foods is beset with difficulties and uncertainties (House, 1999). Hence, micronutrient bioavailability is a confusing and complex issue for human nutritionists that remains a very active research venue for many researchers worldwide (Van Campen and Glahn, 1999). There is no one bioavailability method applicable for all micronutrients or for all circumstances and plant foods (Fairweather-Tait and Hurrell, 1996).

Factors Affecting Micronutrient Bioavailability

Genetic Selection & Production Practices	Other Meal Components	Processing & Preparation	Individual's Characteristics
Nutritional efficiency	Antinutrients	Raw	Age
Fertilizer practices	Promoters	Cooking	Sex
Soil amendments	Protein quality	Fermentation	Ethnicity
Cropping system	Quantity of other nutrients	Malting	Economic status
Variety	Other interacting micronutrients	Extraction	Physiological status
Soil fertility & health	Supplements	Soaking	Nutritional status
Forms in edible portion		Fortificants	Disease status
Antinutrients		Freezing	Education
Promoter substances		Drying	Genetic propensity
Other interacting elements		Polishing	
		Milling	

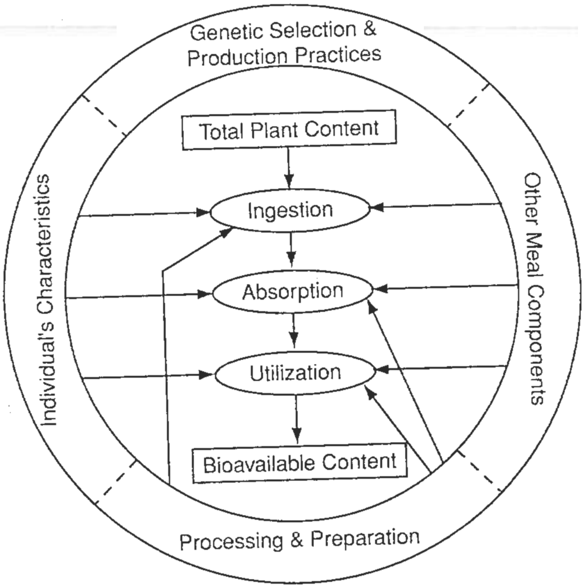

Figure 6 Factors affecting micronutrient bioavailability to humans from plant food sources.

B. BIOAVAILABILITY TECHNIQUES

Various methods have been developed to determine micronutrient bioavailability in plant foods to humans that encompass *in vitro* or *in vivo* models or combinations of both (Van Campen and Glahn, 1999; Wienk *et al.*, 1999). Unfortunately, none of these models is ideal for all foods, nutrients, and circumstances. Ultimately, experiments with human subjects fed enriched staple foods grown locally and consumed in traditional diets under real world situations will determine the actual benefit of enhancing the concentration of micronutrients in staple plant foods to people at risk of developing micronutrient malnutrition. However, such human experimentation is costly and long-term in nature. Therefore, practically speaking, plant breeders must rely on other less costly bioavailability methods to screen relatively large numbers of promising experimental lines. From this preliminary screen, only the most promising ones will be selected for use in conducting human feeding trials under free-living conditions in order to keep expenditures within the permissible limits of available funds.

In our international breeding effort, initial bioavailability screening of promising lines of micronutrient-enriched staple food crops was performed using a rat model (see reference Welch *et al.,* 1974 for detailed information concerning the rat model used). Some researchers consider the rat bioavailability model to be an obsolete model because it is only empirically based (Wienk *et al.*, 1999). Although, quantitatively, rats are far more efficient at absorbing Fe and Zn than humans from plant food sources and sometimes they do not respond as humans do to certain inhibitors such as tea, rats have been used effectively to rank foods with respect to bioavailable Fe and Zn. For example, the effects of some dietary factors, such as ascorbate, soy protein, or bran, on iron bioavailability have been shown to be similar in both rats and humans fed the same dietary sources of Fe, although quantitatively, the actual bioavailability values were considerably higher in the rats (Reddy and Cook, 1991). Importantly, crop lines shown to have relatively low bioavailable amounts of Fe and Zn in rats would, most certainly, have very low bioavailable amounts of these nutrients when fed to humans. However, some lines showing high levels of bioavailable Fe and Zn in a rat model, may not contain highly bioavailable levels of Fe and Zn when fed to humans; thus, the need ultimately to screen the top lines selected from rat models using human trials. While not ideal, for the purpose of initially screening promising lines of Fe- and Zn-dense staple foods, the rat model is a useful tool for ranking promising lines for their bioavailable Fe and Zn content. However, it must not be the ultimate test for determining bioavailable levels of Fe and Zn in micronutrient-enriched staple foods to humans.

Interestingly, for comparative purposes, we screened some promising lines of rice and beans using both a rat model and an *in vitro* human intestinal cell culture technique. The bioavailability rankings obtained with rats were compared to those rankings obtained using a cell culture model (i.e., Caco-2 cell culture). Caco-2 cells were developed from an adenocarcinoma isolated from a human large intestine. Under appropriate culture conditions these cells differentiate into a polarized monolayer of cells having microvilli. Caco-2 cell monolayers are morphologically and physiologically very similar to the layer of mucosal epithelial cells that line the surface of the small intestine and are responsible for most micronutrient-metal absorption from the gut. Similar rankings of the lines tested were obtained for Fe bioavailability using either of these models, giving support for continued use of the rat model in supporting plant breeding efforts. Currently, the Caco-2 cell model does not require radio-labeling of plant foods with Fe radioisotopes which is a distinct advantage over using the rat model which does. However, the human cell culture model has not been developed for use in determining the bioavailability of non-radiolabeled Zn in plant foods. When such a technique is developed for Zn, the human Caco-2 cell model may become the technique of choice for use in screening large numbers of lines for bioavailable Fe and Zn because it is a human cell model and is relatively inexpensive, rapid and free of the need for radioisotope or stable-isotope labeled plants (see Van Campen and Glahn, 1999 for more information on the Caco-2 cell culture model).

C. Intrinsic Versus Extrinsic Labeling of Plant Foods for Bioavailability Determinations

After selecting a technique to determine bioavailable levels of Fe and Zn in plant foods, one is faced with the decision to use either intrinsic or extrinsic labels of these nutrients in meals to be fed to experimental subjects. Commonly, bioavailability methods require that either radioactive or stable isotopes of Fe or Zn be used to label these nutrients in plant foods before their bioavailability can be determined in model systems or in humans. Intrinsic labels refer to the growing of plants in growth media to which the isotopes have been added allowing the plants to absorb them and to incorporate them "naturally" into their metabolites. Extrinsic labels refer to adding inorganic solutions of these isotopes to meals prepared from plant foods after the edible plant portions have been harvested. Extrinsically labeled plant foods are relatively inexpensive to prepare and are easy to use, while obtaining intrinsically labeled plant foods is a more costly, time-consuming and difficult process. Therefore, many researchers have used extrinsic labels to perform bioavailability studies. While extrinsic labels have been shown to produce reliable bioavailability results under many if not most circumstances for several plant foods, they do not always reflect what is found using intrinsic labels. Therefore, the use of intrinsic labels is the only method that assures unequivocal Fe and Zn bioavailability results for all plant food sources when fed to humans in meals containing many interacting factors (House, 1999; Van Campen and Glahn, 1999).

D. Antinutrients in Staple Food Crops

Phytic acid: Plant foods contain various substances that can interfere with the absorption or utilization of Fe and Zn in staple plant foods. Table VI lists examples of antinutrients that are known to be present at high levels in many staple plant foods and their edible products. Among those listed, phytic acid [(myo-inositol hexakis(dihydrogen phosphate)], or its natural product, phytin (a K, Mg salt of phytic acid), has been studied the most because phytin is abundant in edible seeds and grains and forms insoluble precipitates with a number of polyvalent mineral cations (e.g., Ca^{2+}, Fe^{3+}, and Zn^{2+}) *in vitro,* and when added to purified diets. Sodium phytate is known to decrease Fe and Zn absorption in a number of monogastric animal species and in humans (Erdman, Jr. and Poneros-Schneier, 1989; Pallauf and Rimbach, 1997). High dietary Ca accentuates the effects of phytate on Fe and Zn bioavailability, and using Zn-phytate/Zn molar ratios in diets has been reported to be a better predictor of Zn bioavailability than dietary phytate alone. For humans, ratios above 0.5 mol/kg dry diet or 200 m*M*/1000 Kcals may be a cause of concern for Zn nutriture.

Table VI

Examples of Plant Food Substances (i.e., Antinutrients) that can Inhibit the Bioavailability of Nutrients to Humans

Antinutrient	Nutrients affected	Examples of major dietary sources
Phytic acid (i.e., phytin)	Fe, Zn, Mg, Ca	Legume seeds and whole cereal grains
Fiber (e.g., cellulose, hemicellulose, lignin, cutin, suberin, etc.)	Fe, Zn	Whole cereal grain and products
Tannins and other polyphenols	Fe, Zn	Tea, coffee, non-white bean lines, certain sorghum lines
Oxalic acid	Ca	Spinach leaves, rhubarb stems
Hemagglutinins (e.g., lectins)	Fe, Zn	Most legume seeds, wheat grain
Goitrogens	I	*Brassicas* and *Alliums* (e.g., cabbage, broccoli, onion, ect.)
Toxic metals (e.g., Cd, Hg, Pb, Ag)	Fe, Zn	Plant foods from metal-polluted soils (e.g., Cd in rice grain)

There is no doubt that high levels of phytic acid can inhibit the bioavailability of Fe and Zn to people eating staple plant foods such as whole cereal grain products and legume seeds. However, plant foods high in phytin do not always inhibit Fe and Zn bioavailability, and the reasons for the discrepancies are not currently understood (House, 1999). For example, Morris and his cooperators (Morris, 1986; Morris and Ellis, 1982) identified the natural chemical form of Fe in wheat seeds to be monoferric phytate. They then reported that monoferric phytate was relatively highly bioavailable to rats, dogs and humans. Furthermore, in a mineral balance study with human subjects (see Table VII) they compared high-phytate whole wheat bran muffins to dephytinized wheat bran muffins very low in phytate. Meals containing the muffins were eaten daily over the 15 days of the study. Initially, those men given high phytate whole wheat muffins were in negative Fe balance during the first 5 days of the study. After an additional 10 days, the men were in positive Fe balance eating the high phytate bran muffins. Those men eating the dephytinized muffins remained in positive Fe balance over the course of the study. Thus, there appeared to be some change in the men eating the high-phytate muffins that allowed them to become in positive Fe balance after 15 days of consuming the muffins. The reason for this change from negative to positive Fe balance in adult men while still consuming high-phytic acid muffins is still not known.

Some reports concerning the effects of naturally occurring phytin in seeds and grains on Zn bioavailability are confusing and contradictory (Hambidge *et al.*, 1986; House, 1999; Morris, 1986; Welch, 1993). Certainly, soluble salts of phytate when added to purified diets used in animal models or fed to humans do reduce Zn bioavailability. However, other dietary factors, such as Ca and protein

Table VII

Apparent Absorption of Fe by Adult Men Consuming Either Whole Bran Muffins or Dephytinized Bran Muffins for a Period of 15 Days

Whole bran muffin			Dephytinized bran muffin	
5 days	10 days		5 days	10 days
		(mg Fe day^{-1})		
		Subjects 1–5		
−3.0–1.1[1]	2.2–0.4		1.3–2.5	2.8–1.1
		Subjects 6–10		
−0.3–2.5	3.5–0.9		0.0–1.6	1.1–1.8
		All subjects		
−1.6–1.6	2.9–0.7		0.7–1.6	2.0–1.1

Note: Whole bran muffin meals contained 2.0 mg of phytic acid while dephytinized muffin meals contained 0.2 mg of phytic acid.

[1]Intake minus fecal excretion; means–standard deviation (n = 10).

Data from Morris and Ellis (1982).

type, can interact with phytin causing large negative effects of phytin on the bioavailability of Zn. More research is required before all the mechanisms of phytin in reducing the bioavailability of Fe and Zn to humans consuming mixed diets are fully understood. The commonly accepted mechanism for phytate's antinutritive activity, metal-phytate precipitation in the gut, may not be the only way in which phytate changes Fe and Zn bioavailability because phytate and its hydrolysis products can be readily absorbed by mucosal cells in the gut (Sakamoto *et al.*, 1993). Some of these compounds may produce hormone-like reactions within the cells that impact the regulation of nutrient absorption and utilization (Harland and Morris, 1995; Harland and Narula, 1999; Pallauf and Rimbach, 1997).

Some nutritionists are currently promoting the idea that the nutritional quality of staple plant foods could be dramatically improved if genetic means were found to reduce or eliminate antinutrients from these foods. This idea seems logical and sensible on the surface, but further thought leads to some questions that should be addressed before attempts are made to transform staple food crops in this way (Graham and Welch, 1996; Welch, 1993). Many of these antinutrients are major plant metabolites that play important roles in the life of the plant. Removing them from plant seeds may have unforeseen implications for crop productivity, including reduced disease resistance, lower insect and herbivore resistance, lower seed-nutrient stores, and less stress tolerance. Logically, there must be important reasons why plants have evolved the complicated genetic mechanisms required to synthesize, regulate, store, and degrade these types of substances in their reproductive organs.

Recently, low-phytate mutants of staple plant foods have been identified (e.g., low-phytate maize kernels) (Ertl *et al.*, 1998; Sugiura and Raboy, 1999). They were developed for the feed industry to reduce P inputs into monogastric animal and fish rations as well as to reduce P pollution of the environment from pig and chicken manures and fish feces produced when monogastric animals and fish are fed high-phytate rations. While phytate is not hydrolyzed appreciably in the gut of pigs, chickens, and fish, the phytate in the manure of monogastric animals is easily hydrolyzed by soil microbes releasing inorganic P to the environment and causing P pollution of field run-off water and, ultimately, streams, rivers, and lakes. Some have suggested that these types of low-phytate mutants be incorporated in to plant breeding programs to reduce phytate in these food crops thereby lowering the negative effects of phytate on Fe and Zn bioavailability in human populations.

A recent report supports this suggestion (Mendoza *et al.*, 1998). These researchers studied the effects of genetically modified low-phytate maize (LPM) on Fe absorption by humans fed tortillas made from LPM. The LPM tortillas contained only 35% of the phytate found in tortillas made from wild-type maize (WTM). Iron absorption from the LPM was 8.2% of intake while absorption from WTM was significantly lower ($P < 0.001$) at 5.5% of intake. The authors concluded that genetically modified maize lines with the LPM-trait may improve Fe absorption in human populations that are dependent on maize as a staple food. However, this possibility should be critically scrutinized through further research before any attempts are made to incorporate the low-phytate traits into a plant breeding program for staple plant foods to feed people in many developing countries. The needs for further research are discussed below.

Phytin is the primary storage form of P in all higher plant seeds and grains of nutritional importance (Welch, 1986). Phytin is accumulated in globoid crystals in membrane-bound protein bodies of certain cell types within the developing seed, such as those occurring in aleurone cells of the aleurone layer and embryo of cereal grains (Lott, 1984; Mazzolini and Legge, 1981; Ogawa *et al.*, 1979). The phytin deposited within protein bodies as globoid crystals, is associated with the accumulation not only of P, but also of other minerals including K, Mg, Fe, Zn, Cu, Mn, and, in some seed cell types, Ca. Phytin, therefore, plays an important role as a storage pool for mineral nutrient reserve required by the developing embryo within the seed or grain during germination, and during early seedling growth. It contributes to the viability and vigor of the seedling produced (Welch, 1986). Selecting for seed and grain crops with substantially lower phytin content to improve these crops as sources of bioavailable Fe and Zn could have undesirable effects on agricultural production especially in regions having soils of low P status and inadequate trace mineral fertility. Research should be conducted to assure that reducing phytate levels will not have adverse impact on crop production in developing countries which can ill afford to have reduced crop yields or increased production costs to feed their growing populations.

Interestingly, several researchers report that phytic acid (or certain hydrolysis products) may play important roles in lowering chronic disease rates in humans, including certain types of cancer and heart disease. Phytic acid may also perform other beneficial functions related to human health (see text box below) (Harland and Morris, 1995; Pallauf and Rimbach, 1997; Zhou and Erdman, Jr., 1995). Thus, reducing phytate levels in important staple food crops could have adverse effects on human health by increasing cancer risk and other chronic diseases. Therefore, plant scientists should be cautious about trying to modify food crops with respect to their phytin content until further research is forthcoming on this important topic.

Another approach to eliminating the negative effect of phytate in staple plant foods on Fe and Zn bioavailability in human diets has been initiated by some researchers using transgenic techniques. For example, the gene for a heat stable phytase has been identified and isolated from a fungus (*Aspergillus fumigatus*). This fungal gene was incorporated into rice and was expressed in the rice-grain endosperm during grain development (unpublished results presented by Potrykus and co-workers in abstract form at the XVI International Botanical Congress, St. Louis, MO, 1999). According to information in the abstract, when the rice grain carrying the phytase was cooked, all of the phytic acid in the grain was degraded. While this type of genetic modification of rice would improve the nutritional quality of this important staple food with respect to Fe and Zn, it may also increase the risk of developing certain chronic diseases in people consuming rice as a staple food. Hence, more research is needed to determine the overall benefits of phytate to humans before attempting to reduce phytate levels in plant foods.

Phytate or some of its inositol polyphosphate hydrolysis products may:

A. Decrease risk of colon cancer
B. Have chemopreventive and therapeutic actions against cancer
C. Lower serum cholesterol and triglycerides
D. Be a natural antioxidant reducing lipid peroxidation
E. Function in second messenger transduction systems
F. Ameliorate oxyradical-induced myocardial ischemica/reperfusion damage
G. Help prevent renal calculi from forming, i.e., reduce risk of kidney stones
H. Increase Cu bioavailability from foods

E. Promoter Substances

Even in the presence of the antinutrients, certain dietary substances (i.e., promoter substances; see Table VIII) can enhance the bioavailability to humans of micronutrients, including Fe and Zn, in meals containing plant foods (Ashmead and

Table VIII

Examples of Dietary Substances (i.e., Promoters) that can Enhance the Bioavailability of Nutrients to Humans from Plant Foods

Substance	Micronutrient promoted	Common dietary sources
Some organic acids (e.g., ascorbate, fumarate, malate, citrate)	Fe and/or Zn	Various fresh fruits, vegetables
Phytoferritin (i.e., plant ferritin)	Fe	Legume seeds, leafy vegetables
Some free amino acids (e.g., methionine, cysteine, histidine, lysine)	Fe and/or Zn	Animal meats (e.g., beef, pork, fish)
The "meat factor" (polypeptides rich in cysteine?)	Fe, Zn	Animal meats (e.g., beef, pork, fish)
Long-chain fatty acids (e.g., palmitic acid)	Zn	Human breast milk
Fats and lipids	Fat soluble vitamins (e.g., vitamin A)	Animal fats, vegetable oils
Se	I	Sea foods, tropical nuts from plants grown on high-Se soils
Zn	Fat soluble vitamins (e.g., vitamin A, vitamin E)	Animal meats
β-carotene (provitamin A carotenoids, vitamin A)	Fe	Green and orange vegetables, red palm oil, yellow maize
Vitamin E (α-tocopherol)	Vitamin A	Vegetable oils, green leafy vegetables
Riboflavin (e.g., falvin mononucleotide, favin adenine dinucleotide)	Fe and Zn	Liver, beef, cheese, broccoli
Inuline (oligofructose, β-2,1–fructo-oligosaccharides)	Ca	Chicory, Jerusalem artichoke

Christy, 1985; Gordon and Godber, 1988; Michaelsen and Friis, 1998; Mulvihill and Morrissey, 1998a, 1998b; Politz and Clydesdale, 1988; Welch and House, 1995). Many of these promoter-substances are normal plant metabolites and modest increases in their concentrations may not result in adverse effects on plant growth and development. Usually, these factors are rich in certain foods such as animal meats and many types of fruits and vegetables, but are at relatively low levels in staple foods such as cereal grains and legume seeds. Increasing promoter concentrations in staple food crops by genetic manipulation is an attractive possibility if we know the chemical identity of the promoter substance and the genes responsible for its expression. Taking this approach would eliminate many of the concerns related to decreasing antinutrients in these crops discussed above (Graham and Welch, 1996). Breeding for increased promoter substances in plant foods is an important opportunity for plant scientists to improve the nutritional quality of staple foods with respect to some micronutrients including Fe and Zn. Additionally, producing transgenic plants via modern genetic engineering techniques that express more of these substances in edible portions of food crops is an important area for additional research and development in plant nutritional genomics. Indeed, Potrykus's research group in Switzerland, that developed the transgenic rice line containing a heat stable phytase (see discussion above), over expressed a rice gene for metallothionine in the same rice line increasing the level of this cysteine-rich protein in the grain-endosperm by about 25%. They believe that doing so would improve the bioavailability of Fe in the rice grain to humans although there is no animal or human data published to date to substantiate this claim.

Ascorbic acid: Among the promoter substances listed in Table VIII, ascorbic acid (vitamin C), the "meat factor" and β-carotene (a vitamin A precursor) have received the most interest scientifically. Ascorbic acid is thought to enhance the absorption of Fe from plant foods via forming Fe(III) complexes and by reducing Fe^{3+} to the more soluble and bioavailable Fe^{2+} valence state (House, 1999). While ascorbic acid markedly increases nonheme Fe absorption in humans, relatively long-term dietary supplementation with ascorbate had less effect on Fe bioavailability than expected based on experiments with single meals (Hunt *et al.*, 1994). Consuming ascorbate-rich foods has been reported to counteract phytate inhibition of Fe absorption from wheat rolls (Hallberg *et al.*, 1989). Another effect of ascorbate intake was to prevent the dose-dependent inhibition of Fe absorption by polyphenols and phytic acid in maize bran (Siegenberg *et al.*, 1991) and to ameliorate the adverse effects of phytate in rice starch on Fe bioavailability to humans (Tuntawiroon *et al.*, 1999). Therefore, increasing the ascorbate levels in plant foods genetically would help alleviate the negative effects of phytate and polyphenols in staple foods on Fe bioavailability and at the same time, enhance these foods as sources of the essential nutrient, vitamin C. Such efforts should be encouraged.

The meat factor: The chemical identity of the "meat factor" promoter has not been discovered, but it appears be related to cysteine-rich polypeptides in muscle proteins from animal sources (Gordon and Godber, 1988; Kapsokefalou and Miller, 1995; Mulvihill and Morrissey, 1998b, 1998a; Politz and Clydesdale, 1988; Welch and House, 1995). Some studies have shown that cysteine and cysteine-rich polypeptides enhanced the bioavailability of Fe to monogastric animals and humans (Taylor *et al.*, 1986); these findings support the contention that the "meat factor" is a polypeptide rich in this amino acid (Welch and House, 1995). Others have reported that methionine in addition to cysteine can promote Zn bioavailability (House *et al.*, 1996; House *et al.*, 1997; Welch and House, 1995). Greater efforts should be applied to identifying the chemical form and mechanisms of action of the "meat factor". Once identified, ways should be found to incorporate this promoter into staple plant foods containing high levels of antinutrients, such as phytic acid. This would improve the bioavailability of Fe and Zn from these important plant foods, taking full advantage of any genetic enrichments made in Fe and Zn levels in these staples through plant breeding.

Phytoferritin: Ferritin is a major storage form of cellular Fe in animals, plants, and certain bacteria that is also involved in controlling cellular Fe homeostasis (Andrews *et al.,* 1992). In humans, serum Fe-ferritin stores are one factor used in determining Fe status of individuals (Dallman, 1990). Ferritin molecules are multimeric proteins that sequester up to 4500 atoms of Fe as hydrous ferric oxide-phosphate inside a protein coat having a molecular weight of about 450 kDa (Briat, 1996). The apparent bioavailability of Fe from ferritin to rats has been reported. Iron provided as ferritin from horse spleen in a Fe-deficient semi-purified diet was able to fully recover rats from iron deficiency-induced anemia, demonstrating that the Fe in ferritin was bioavailable to rats. Additionally, rats fed soybean meal containing phytoferritin were able to fully recover from Fe deficiency anemia. However, the recovery could have resulted not from phytoferritin but from other forms of Fe present in the soybean meal (Beard *et al.,* 1996).

It has been proposed that significantly increasing phytoferritin levels in major food corps could contribute greatly to decreasing Fe deficiency globally (Theil *et al.,* 1997). Interestingly, Goto *et al.* (1999) have enriched the grain-Fe content of a rice line by as much as 300% by transferring the entire genetic coding sequence for soybean phytoferritin into the rice line using *Agrobacterium*-mediated transformation. They used a grain-glutelin storage protein promoter (*GluB-1*) that allowed the expression of the gene in the rice grain endosperm. If the Fe in rice-grain phytoferritin is found to have a high bioavailability to humans, and if it can be shown that phytoferritin has no negative effects on plant growth or harmful effects in humans, then this technique should be considered for use in developing other Fe-dense staple food crops.

Plant nonsymbiotic hemoglobin: The Fe in hemoglobin from animal meat sources is relatively highly bioavailable to humans compared to nonheme forms from plant foods. Additionally, heme-Fe absorption by gut cells is not as affected by several antinutrients such as phytate and polyphenols as is nonheme Fe. Furthermore, the regulation and mechanisms of heme-Fe absorption by gut cells are different from that of nonheme Fe (House, 1999). For these reasons, several laboratories are currently trying to express hemoglobin (such as symbiotic leghemoglobin from soybean nodules) synthesis in grains of cereal crops in order to improve these crops as sources of Fe for people (personal communication, David Garvin, U.S. Plant, Soil and Nutrition Laboratory, Ithaca, NY). Expressing the gene for the synthesis of symbiotic leghemoglobin in staple seeds and grains is one approach that could be used. It will be necessary to assure that this form of Fe once expressed in seeds and grains does not produce harmful effects on seed viability and seedling vigor, and that the leghemoglobin produced provides bioavailable Fe for humans in a safe and effective form.

Interestingly, within the last 4 years evidence has been reported showing that nonsymbiotic hemoglobin proteins are widely found in the plant kingdom (Arredondo-Peter *et al.*, 1997; Hill, 1998). For example, in barley, hemoglobin biosynthesis was induced under low oxygen tensions and was regulated by ATP or by actions of ATP on metabolism. Additionally, hemoglobin expression was demonstrated to be a normal consequence of barley seed germination (Duff *et al.*, 1998). By using transformed maize cells, researchers were able to show that hemoglobin acted to improve the energy status of the cells when grown under low-oxygen stress conditions (Sowa *et al.*, 1998). These authors suggested that nonsymbiotic hemoglobins act in plants to maintain energy status of plant cells growing in low-oxygen environments by promoting glycolytic flux via NADH oxidation and promoting substrate-level phosphorylation. Thus, hemoglobin proteins may play important functions in plant growth and in low-oxygen stress resistance.

Arredondo-Perter *et al.* (1997) reported the cloning and analysis of two nonsymbiotic hemoglobin genes (*hb1* and *hb2*) from rice. They found at least three copies of the gene coding hemoglobin in rice DNA. These genes are expressed in the roots (*hb1*) and the leaves (both *hb1* and *hb2*) of rice. The cDNA for rice HB1 was expressed in *Escherichia coli* and the recombinant hemoglobin (rHB1) had a high affinity for O_2. Further research identifying the genes responsible for hemoglobin protein synthesis in plants is needed in order to identify likely candidate genes to use in transforming staple plant foods.

Even though the exact metabolic function(s) of nonsymbiotic plant hemoglobin is (are) still to be elucidated, the natural occurrence of nonsymbiotic hemoglobin in plants is of interest because these genes that encode hemoglobin synthesis could be over expressed in edible portions of crop plants using modern molecular biology techniques and appropriate gene promoters for seed-endosperm expression. If successful, this could lead to dramatic improvements in the bioavailability of Fe in staple plant foods that would be less affected by the presence of antinutrients in these foods. Still, the bioavailability of Fe in nonsymbiotic hemoglobin proteins and the safety to humans of consuming this form of Fe will have to be determined in future studies to assure that such changes will have a significant and safe impact on human Fe status and health.

A cautionary note: Some groups of people are concerned about increasing the bioavailable levels of Fe in staple plant foods. Idiopathic hemochromatosis (a relatively rare disease affecting about 1 per 500 to 1 per 1000 people) is characterized by uncontrolled Fe absorption and Fe overload disease in individuals afflicted with this genetic disorder which can result in death from Fe toxicity in midlife if not treated (Barisani *et al.*, 1996). Additionally, homozygous thalassemia, a relatively rare hereditary hemolytic anemia, requires numerous blood transfusions for survival in individuals expressing this gene. Increased Fe intake in these individuals adds an undesirable additional Fe load to their body. Both idiopathic hemochromatitic individuals and individuals being treated for homozygous thalassemia want to avoid extra iron in their diets (Dallman, 1990). However, iron deficiency anemia afflicts over 2 billion people globally, having serious impacts on human health, cognitive function, and productivity. It would seem prudent and necessary to improve the Fe content of staple foods to deal with this immense Fe-deficiency crisis in the general population, keeping in mind that relatively few people will be adversely affected by doing so. The profound benefits to massive numbers of people outweigh the risks to relatively few individuals who can easily avoid such foods.

β-carotene: Recently, several reports by Layrisse and colleagues (Garcia-Casal *et al.*, 1998; Garcia-Casal and Layrisse, 1999; Layrisse *et al.*, 1997; Layrisse *et al.*, 1998) indicated that fortifying cereal-based diets with vitamin A or β-carotene and Fe(II)-fumarate enhanced the bioavailability of the Fe to humans dramatically (e.g., β-carotene increased Fe bioavailability more then three fold in rice-based meals and more then 1.8-fold in wheat and corn-based meals). The researchers also suggested that the effect of β-carotene and vitamin A on promoting the Fe bioavailability in a meal containing high levels of phytate and polyphenols was the result of complexation of Fe(III) with β-carotene or vitamin A in the gut during digestion. They suggested that this prevented the precipitation of Fe in the gut by phytate or polyphenolics from foods in the meals used in their study. If true, β-carotene and vitamin A may not promote Fe bioavailability in plant foods that are low in the antinutrients, phytic acid, and polyphenolics. Preliminary results from our laboratory indicate that this may be the case because Fe bioavailability to rats fed intrinsically Fe^{59}-labeled cauliflower curds from high and low β-carotene cauliflower lines (containing negligible amounts of phytate and polyphenols) was not related to β-carotene levels in the cauliflower curds (unpublished results). Highest and lowest levels of bioavailable Fe were found for white-curded cauliflower lines, neither of which had measurable β-carotene levels.

Potrykus and his colleagues (see discussion above) also reported producing yellow endosperm transgenic rice grain containing high levels of β-carotene along with the previously discussed elevated Fe levels, the heat-stable phytase and elevated metallothionein levels. They added three genes to the rice line to accomplish this, two from daffodil and one from the bacterium *Erwina uredovora* (Ye *et al.*, 2000). According to their news release, the resulting transgenic rice line synthesized enough β-carotene in its grain-endosperm to color the grains distinctively.

Importantly, experiments should be conducted to determine if human nutriture with respect to vitamin A and iron status is improved significantly when fed the transformed rice grain produced by Potrykus and his associates. If this type of transgenic rice grain is found to improve the Fe and vitamin A status of targeted populations at risk of developing deficiencies of these micronutrients, attempts should be made to perform similar genetic modifications of other important plant foods including wheat, maize, and beans.

F. Bioavailability Issues Concerning Human Studies

It is virtually impossible to design human clinical studies under laboratory conditions to determine the micronutrient bioavailability in micronutrient-enriched lines of staple plant foods that give unequivocal conclusions applicable to free-living populations in various environments. There is no one technique that is universally applicable for all micronutrients and all crops, or that can encompass all the factors known to affect bioavailability. Clinical trials can be performed using enriched lines, but these types of controlled experiments performed under laboratory conditions cannot include all the variables that can affect micronutrient bioavailability to humans (see Fig. 6). Just carrying out clinical studies might lead to conclusions that are misleading or even erroneous, either showing or not showing significant nutritional benefits to subjects eating the enriched lines under the conditions of the laboratory experiment. This could lead either to the elimination or the inclusion of an enriched line for future development, or even to the conclusion that breeding for micronutrient-enriched staples is without value. For this reason, the only ways to ascertain with certainty if micronutrient-enriched lines of staple food crops actually improve human nutriture is to carry out long-term feeding trials comparing the enriched lines with traditional lines of staple foods. Unfortunately, these types of studies are expensive and long-term in nature. They must be performed with groups of people that have been rigorously selected in ways that will assure reliable results. Some examples of factors to consider in developing Fe and Zn feeding trials using human subjects are discussed below.

Iron bioavailability feeding trials: Selecting human subjects for field trials to determine the efficacy of Fe-enriched staple foods for improving Fe status must meet certain criteria. They must assure that the subjects to be studied are dependent on the staple food as a major constituent of their diet, that the subjects are free of disease, inflammatory conditions, parasites, and that their body stores of iron are significantly depleted (e.g., serum-ferritin values are $<12\ \mu g\ L^{-1}$, transferrin saturation $< 16\%$ for adults), but not so depleted that they are severely iron deficiency anemic (e.g., hemoglobin levels $< 12\ g\ L^{-1}$) (Dallman, 1990). Additionally, data concerning their typical diets, food preparation techniques and household food distribution patterns must be collected (i.e., a full knowledge of their food system must be acquired). The staple lines to be studied should be grown in the region where the study will take place and by the accepted means of cultivation. The length of the study will depend on the amount of Fe enrichment obtained in the staple food produced, lasting as long as several months to a year or more. Traditional lines relatively low in Fe should be included as a control group. A dietary supplement should be provided that assures that all subjects are adequate in all micronutrients except Fe during the course of the study. Subjects should be monitored closely to ascertain compliance to the study protocols, to determine how much of the staple food they consume, how it is prepared and distributed among family members, and what foods are consumed with the test line. Fe status should be determined before the study ensues, during the study, and when the study culminates. Anthropometric data (e.g., height, weight, arm circumference, and triceps skinfold thickness) should also be collected during the course of the study.

Feeding trials for zinc bioavailability: Most of the information needed to conduct meaningful feeding trials with Fe also applies to Zn (Gibson and Ferguson, 1998). Normally, rich dietary sources of bioavailable Fe (i.e., animal meats) are also rich sources of bioavailable Zn, and people at risk of developing Fe deficiency are usually at risk of developing Zn deficiency. Unfortunately, determining the Zn status of an individual cannot be performed using an available clinical procedure unless the person is severely Zn deficient because no reliable clinical test exists to determine marginal Zn status in humans. Plasma-Zn is the most frequently used method to determine Zn status, but a variety of factors affect plasma-Zn leading to misinterpretation of Zn status. A combination of Zn deficiency characteristics (e.g., growth retardation, delayed sexual and skeletal maturity, skin lesions such as orificial and acral dermatitis, diarrhoea, alopecia, behavioral changes) and severe hypozincemia makes the detection of severe human Zn deficiency relatively easy (Hambidge *et al.*, 1986; World Health Organization, 1996). In the severe Zn-deficient state, concentrations of Zn in the plasma are usually $< 0.4\ \mu g\ mL^{-1}$ and many times $< 0.2\ \mu g\ mL^{-1}$ and under moderate deficiency they fall between 0.4–0.6 $\mu g\ mL^{-1}$. In Zn-adequate individuals plasma Zn values lie within the range of 0.65–1.10 $\mu g\ mL^{-1}$. Regrettably, in moderate Zn-deficient individuals, many features of Zn deficiency are nonspecific, and factors other than Zn deficiency can cause low Zn-plasma levels. Identification of mild Zn deficiency in humans is very difficult because plasma Zn levels can be within the normal range and other characteristics of Zn deficiency are not specific to this disease. Currently, the only sure way to determine mild Zn deficiency in humans is through determining the response of individuals to Zn supplementation of their diet (Hambidge *et al.*, 1986). Therefore, any human feeding trial conducted to determine the effects of Zn-enriched staple foods on human Zn status will have to rely primarily on anthropometric data in children and associated Zn-deficiency-related diseases and characteristics. Selecting subjects with low Fe stores will aid in finding individuals with low Zn status, but cannot guarantee that Zn-deficient subjects will be included in the study. Zinc status is important in the selection criteria because individuals with adequate Zn status down regulate their absorption of Zn from food and may not respond to Zn-enriched staples to the extent that Zn-deficient individuals would (Cousins and Hempe, 1990).

Paik *et al.* (1999) recently published a paper indicating that serum extracellular superoxide dismutase can be used as a functional indicator of marginal Zn deficiency in humans. If this method proves to be reliable after further testing, a clinical method to determine mild Zn deficiency in humans may become available. Other recent papers have also reported that Zn clearance tests may be diagnostic of marginal Zn deficiency in children of small stature (Kaji *et al.*, 1998). Further, mean plasma zinc concentration may be a useful indicator of population Zn status for children in low-income nations despite the high prevalence of common childhood infections in these children (Brown, 1998).

VIII. MARKETING STRATEGIES, ECONOMIC AND SOCIAL CONSTRAINTS, COST-BENEFIT ANALYSIS

A. Food Demand Patterns

An underlying cause of and fundamental constraint to solving the micronutrient malnutrition problem is that nonstaple foods, particularly animal products, tend to be the foods richest in bioavailable micronutrients, which the poor in developing countries cannot afford. Their diets consist mostly of staple foods, primarily cereals. In fact, per capita, direct consumption of staple foods in the aggregate varies little by income level. For the poor, these staple foods already are primary sources of what micronutrients, particularly minerals, they are able to consume.

This is demonstrated by food intake data shown in Tables IX and X for survey

Table IX

Food Expenditures Per Capita Per Week and Percent Contributions to Calorie and Iron Intakes For A Survey Population in Bangladesh

Food group	Poorest 20% of surveyed households	Richest 20% of surveyed households	Average for all surveyed households
	Percent contribution to household food expenditures		
Rice, wheat	69	54	62
Meat, fish	9	19	14
Other foods	22	27	24
Total	100	100	100
	Percent contribution to household calorie intakes		
Rice, wheat	87	81	84
Meat, fish	1	4	2
Other foods	12	15	13
Total	100	100	100
	Percent contribution to household iron intakes		
Rice, wheat	55	43	51
Meat, fish	3	6	5
Other foods	42	52	45
Total	100	100	100

From Bouis (1999).

Table X

Food Expenditures Per Capita Per Week and Percent Contributions to Calorie and Iron Intakes For A Survey Population in the Philippines

Food group	Poorest 20% of surveyed households	Richest 20% of surveyed households	Average for all surveyed households
	Percent contribution to household food expenditures		
Maize, rice	45	24	33
Meat, fish	28	39	32
Other foods	27	37	35
Total	100	100	100
	Percent contribution to calorie intakes		
Maize, rice	84	70	79
Meat, fish	4	10	6
Other foods	12	20	15
Total	100	100	100
	Percent contribution to iron intakes		
Maize, rice	43	30	36
Meat, fish	18	36	25
Other foods	40	34	39
Total	100	100	100

From Bouis (1999).

populations in Bangladesh and the Philippines, respectively.[1] Average incomes in these households range from U.S. $45 per capita per year in the poorest 20% of households to $250 in the richest 20% of households. Thus, they are typical of the middle to lower-end of the income distribution in rural areas of these countries.

The first priority for these poor households in terms of food purchases is to obtain calories to satiate hunger. The most inexpensive sources of calories are food staples, rice and wheat in the case of Bangladesh and maize and rice in the case of the Philippines. Once a critical intake of calories is acquired from inexpensive food staples, as incomes increase consumers purchase nonstaple foods at the margin, particularly animal products and fruits, and to some extent substitute more expensive but more preferred food staples for inexpensive staples.[2]

[1] A detailed discussion of how these data were collected and patterns of food consumption for these populations is provided in Bouis and Novenario-Reese (1997) and Bouis and Haddad (1990) for Bangladesh and the Philippines, respectively. The Bangladesh survey population is somewhat poorer than the Philippines survey population. One half of the Bangladesh survey population was drawn from distressed areas.

[2] A model of food characteristics which drive patterns of consumer food purchases is presented in Bouis (1996). There is disagreement in the economics literature as to whether aggregate food staple intakes increase rapidly with income in developing countries (Subramanian and Deaton, 1996) or not (Bouis, 1994).

Not only are food staples poor (low-density) sources of trace minerals, but antinutrient (e.g., phytate) levels are high which may reduce the bioavailability of the trace minerals consumed. Nevertheless, for poor populations such as those represented in Tables IX and X, food staple consumption so dominates diets (their low incomes preclude the consumption of desired levels of non-staple foods) that primary food staples provide in the range of 40–55% of total Fe intakes for lower income households shown.

If a single food staple provides 50% of total Fe intakes for a poor population (e.g., for rice in Bangladesh), then doubling the Fe density in that food staple will result in a 50% increase in total Fe intakes, and tripling the Fe density will mean a doubling of total Fe intakes.

A strength of a plant breeding approach which focuses on food staples, is that it is based on existing consumer behavior. The poor consume large amounts of food staples on a daily basis. If a high proportion of the domestic production of food staples can be provided by nutritionally improved varieties, nutritional status can be improved without resorting to programs that depend on behavioral change.[3] Trace minerals constitute such a small physical part of the grain (at most a few dozen parts per million) that increasing density is not expected noticeably to change consumer characteristics (e.g., appearance, taste, odor, texture, cooking qualities).

For the lower income households in Tables IX and X, iron intakes for women range between 50–75% of recommended daily allowances. Despite well-known difficulties with determining useful benchmarks for recommended daily allowances of Fe, it would seem evident that a 50% increase in intakes of bioavailable Fe would be of considerable benefit to anemic women with such low Fe intakes. Nevertheless, human studies still need to be undertaken to measure effects of increased Fe (or Zn) density in food staples on Fe (or Zn) status and consequent improvements in health and productivity.

Similar arguments apply to those staples in which provitamin A content may be enriched by plant breeding (wheat, maize, and cassava, for example). Some differences apply when compared with trace minerals. First, no agronomic advantages accrue to higher provitamin A content, so that high density will need to be bred into varieties that are otherwise high yielding. Second, the color of the final food product may change so that consumers may need to be educated as to the improved nutritional content. If education programs are successful, the color change becomes an advantage in that it identifies those particular varieties of superior nutritional quality.

[3]Likewise no behavioral change is required of farmers, if nutritionally-improved varieties have unique agronomic advantages on trace mineral deficient soils, or if these traits are incorporated into highly profitable varieties. Profits then motivate farmers to adopt and produce these nutritionally-improved varieties.

B. Cost-Benefit Analysis

If plant breeding costs substantially more per benefit received than existing interventions to improve human nutrition (e.g., supplementation and fortification programs), then it makes no sense to pursue such a strategy. The analysis summarized below shows that plant breeding is highly cost-effective. The underlying factors resulting in high benefit-cost ratios for a plant breeding strategy are easy to understand. First, breeding for trace mineral dense seeds has a double benefit—it can improve human nutrition and at the same time it can improve plant nutrition as discussed in Section VI. Existing interventions address only human nutrition—they have no direct effect on agricultural productivity as well. Second, once the initial investment is made in developing a more nutrient-dense genotype, the plants in some sense "fortify themselves." There is no need to undertake and enforce legislation to fortify specific foods. Costs of adding the fortificant to the food vehicle during processing are unnecessary. The only drawback to pursuing a plant breeding strategy is the lag time between initiation of breeding research and the point at which improved varieties become available after being adopted by farmers. No benefits accrue during this period that may range anywhere between 5 and 10 years on average.

1. Costs of Plant Breeding

To obtain a rough estimate of plant breeding costs, the example of the CGIAR Micronutrients Project may be used. The general objective over 5 years has been to assemble the package of tools that plant breeders will need to produce mineral and vitamin-dense cultivars. The target crops are wheat, rice, maize, *Phaseolus* beans, and cassava. The target micronutrients being studied are Fe, Zn, and vitamin A. For these crops and nutrients, this project is conceived as a ***prebreeding*** study to determine:

1. The range of genetic variability available for exploitation by future breeding programs;
2. The stability of expression of the high-density trait across environments (G*E)
3. The bioavailability of the micronutrients contained in the grain (or seeds or other storage tissue) of the best selections;
4. The genetics and physiology/biochemistry of the selected traits;
5. Screening protocols for use in subsequent breeding programs.

The plant breeding effort can be seen as a two-stage process. The first 5-year phase primarily involves research at central agriculture research stations, at an estimated $2 million per year for research on all five crops. During this initial phase, promising germplasm is identified and the general breeding techniques are developed for later adaptive breeding.

During the second phase, the research needs to shift to national agricultural research centers. Total costs and duration of this second phase are difficult to estimate, but will depend on the number of countries involved and the number of crops worked on in each country. Certainly, the annual cost for each country should not be more than the $2 million per year per environment, estimated for the first phase.

2. Benefits to Improved Human Nutrition

The World Bank (1994) estimated that at the levels of micronutrient malnutrition existing in South Asia, 5% of gross national product is lost each year due to deficiencies in the intakes of just three micronutrients: Fe, vitamin A, and iodine. For a hypothetical country of 50 million persons burdened with this rate of malnutrition, deficiencies in these three nutrients could be eliminated through fortification programs costing a total of $25 million annually, or 50¢ per person per year. The monetary benefit to this $25 million investment is quite high in terms of increased productivity—estimated to be $20 per person per year, or a fortyfold return on an investment of 50¢. These benchmark numbers will be used below as a basis of comparison with the benefits of a plant breeding strategy.

3. Calculation of Benefit-Cost Ratios

Calculation of benefit-cost ratios inherently requires making a number of assumptions. In order to tie the assumptions made here to a concrete example, it will be helpful to use the country of Turkey as a case in point. The analysis will focus specifically on the problem of wheat production on Zn-deficient soils in Turkey and its relationship to human nutrition.

Using numbers rounded off for illustrative purposes, about 10 million ha of wheat are harvested each year in Turkey. Average yields are about 2 tons ha^{-1}, giving a production of 20,000 million kg for a population of 60 million people. International trade in wheat is negligible, giving a per capita annual availability of 330 kg, of which about two-thirds is directly consumed as wheat and bread products (FAO, 1995). This establishes that wheat is the primary food staple consumed.[4]

In deriving benefit-cost estimates, it will be useful to make assumptions that will likely ***understate*** benefits and ***overstate*** costs, as discussed below.

About one-half of the 10 million ha harvested to wheat are planted on Zn-deficient soils (Cakmak, 1999). H.-J. Braun (personal communication) has estimated that development of Zn-efficient varieties (the seeds of which are more viable and

[4]220 kilograms per capita per year times a milling rate of 0.75 times 3,540 calories per kilogram of wheat flour, implies a daily per capita consumption of 1600 calories from wheat and bread products.

vigorous as discussed in Section VI) will allow a lowering of seeding rates from 250–150 kg on these 5 million ha. A savings of 80 kg ha^{-1} in seed is assumed below.

Zn-efficient varieties with Zn-dense seeds are higher yielding in Zn-deficient soils for reasons discussed in Section VI. Data from Cakmak (1999) show that wheat yields for present (non-efficient) genotypes be increased by between 1–2 t ha^{-1} on Zn-deficient soils through applications of Zn fertilizers. In the benefit-cost analysis, an increase in yield of 300 kg (about 20% of the possible yield increase of 1.5 t ha^{-1} suggested by the data for Zn fertilizer applications) is assumed for improved Zn-efficient genotypes grown on Zn-deficient soils.[5]

It is assumed that Zn-efficient cultivars eventually are adopted on a maximum of only 4 million out of 5 million ha. This is 40% of total area harvested for all wheat production in Turkey. It is assumed that these improved cultivars are consumed by the equivalent of 20 million people, or one-third of the population.[6]

Between 1993 and 1996, international prices of wheat varied between \$120 and \$263 t^{-1} (FAO, 1997). A value of \$125 t^{-1} is used in the benefit-cost analysis here.[7]

Although there is increasing evidence that Zn deficiencies are a major public health problem in developing countries (Gibson, 1994), no estimates of the benefits of Zn intervention programs exist simply because Zn fortification and supplementation programs are not yet in place. The World Bank analysis of a set of comprehensive fortification programs for three nutrients provides an estimate of benefits of \$20 per person per year, or about \$7 benefit per person per nutrient deficiency eliminated.

For lack of a basis for a more precise estimate, the benefit-cost analysis here simply assumes a benefit of only \$1 per person equivalent consuming the Zn-dense improved wheat cultivars.[8] As discussed in Section VIII.A, Zn densities in food

[5]It may be that returns to research on developing zinc-efficient varieties are higher in Turkey than other countries that have zinc-deficient soils. However, as will be explained below, ***all*** the central costs incurred by the CGIAR Micronutrient Project are assigned only to benefits realized in Turkey and not elsewhere. If benefits in Turkey alone pay for (justify) this central research cost, then spinoff benefits from this central research may be, in some sense, thought of as a free good to other countries. These other countries, of course, will still have to incur country-specific research costs for adaptive breeding to realize these benefits.

[6]As with any fortification program, in practice it will not turn out that some persons will consume only the nutritionally-improved wheat varieties while all other persons will consume none of the nutritionally-improved varieties. However, four million hectares planted to nutritionally-improved varieties could produce enough wheat to meet the total demand of 20 million persons, even if one-third of production goes to other uses (e.g., seed).

[7]The low output price, then, takes into account any increase in production costs, say for harvesting and transport. Also, prices quoted are f. o. b. so that farmgate prices will be lower. However, seeding costs will be lower. Also, trace mineral efficient varieties are more disease resistant and drought tolerant (see section VI), requiring fewer fungicides and less irrigation. No calculation is made in the cost-benefit analysis to account for these savings.

[8]The actual benefit may be much higher than \$1. A conservative assumption is made in keeping with the strategy here of understating benefits and overstating costs.

staples and other foods are similar to those of Fe, and so if Zn densities in wheat seeds were doubled and percent bioavailable Zn were to remain constant, this could yield a benefit derived from a daily increase of 50% in the intake of bioavailable Zn.

Turning to costs, it is initially assumed that all first-phase central research costs (for ***all*** five crops for ***all*** three nutrients—$10 million in total over 5 years) accrue to only one country, Turkey, and that this research is ***very narrowly successful***—only for Zn for wheat. Second phase costs (over 5 years) for Turkey are also assumed to be $10 million, again successful only for Zn for wheat. Maintenance breeding costs are assumed to be $200,000 per year after adaptive breeding (phase 2) is completed.[9]

A benefit-cost analysis, shown in Table XI, is undertaken applying these assumptions. Note that no benefits are derived from plant breeding research until year 11 of the analysis, after all adaptive breeding is completed. Improved varieties are assumed to be adopted on 0.8 million ha, 1.6 million ha, 2.4 million ha, 3.2 million ha, in years 11 through 14 respectively, and on 4.0 million ha thereafter.

All benefits and costs are expressed in terms of *present value*.[10] A discount rate of 12% is used (see Gittinger, 1982, p. 314). Because of the long lag times between investment and benefits for agricultural research, benefits are discounted by 80–90% in years 15–20 of the analysis.

Expressed in present values, costs are about $13 million and benefits $274 million, giving a benefit-cost ratio of over 20, which is quite favorable despite the very conservative assumptions made and despite the long time lag between investments and benefits.

In Table XI, the present value of central research costs ($8.1 million) constitutes 62% of total costs. Although the results of this central research may be applied in countries (developing and developed) all over the world, all costs are applied to Turkey. Also in Table XI, agronomic benefits of the improved seeds account for 90% of the benefits, partially due to the conservative assumptions made as to nutritional benefits.

Table XII undertakes an analysis of the sensitivity of benefit-cost ratios to some

[9]The benefit-cost analysis is not sensitive to this particular assumption due to the fact that these expenses are incurred far enough in the future that the present value is a low percentage of current costs.

[10]The intuition behind concept of present value may be explained by asking: which has more value, $1 today or $1 a year from now? $1 today is worth more in that it could be deposited in a savings account so that it might be worth $1.05 next year, depending on the interest rate. Applying the same logic backwards in time, a $1 benefit next year might be worth only 95 cents today, depending on the *discount* (interest) rate used. Due to compounding of discount rates over a number of years, it is easily seen that the *present value* of a $1 benefit realized ten or more years in the future is a low percentage of $1. In general, if the present value of benefits of a project is greater than the present value of costs (a benefit-cost ratio greater than 1), the project is a sound investment, assuming that an appropriate discount rate has been used.

Table XI

Benefit-Cost Analysis for Breeding for Zinc-Dense Wheats in Turkey

Year	Investment cost: In current dollars[a]	Investment cost: Factor for computation of present value[b]	Investment cost: Present value	Fortification through plant breeding: No. of persons affected (millions)	Fortification through plant breeding: Nutrition benefit	Fortification through plant breeding: Benefit of reduced seeding	Fortification through plant breeding: **Benefit of higher plant yields**	Total benefits: Current dollars	Total benefits: Present value
0	2.0	1.000	2.00	—	—	—	—	—	—
1	2.0	0.893	1.79	—	—	—	—	—	—
2	2.0	0.797	1.59	—	—	—	—	—	—
3	2.0	0.712	1.42	—	—	—	—	—	—
4	2.0	0.636	1.27	—	—	—	—	—	—
5	2.0	0.567	1.13	—	—	—	—	—	—
6	2.0	0.507	1.01	—	—	—	—	—	—
7	2.0	0.452	0.90	—	—	—	—	—	—
8	2.0	0.404	0.81	—	—	—	—	—	—
9	2.0	0.361	0.72	—	—	—	—	—	—
10	0.2	0.322	0.06	—	—	—	—	—	—
11	0.2	0.287	0.06	4.0	4.0	8.0	30.0	42.0	12.05
12	0.2	0.257	0.05	8.0	8.0	16.0	60.0	84.0	21.59
13	0.2	0.229	0.05	12.0	12.0	24.0	90.0	126.0	28.85
14	0.2	0.205	0.04	16.0	16.0	32.0	120.0	168.0	34.44
15	0.2	0.183	0.04	20.0	20.0	40.0	150.0	210.0	38.43
16	0.2	0.163	0.03	20.0	20.0	40.0	150.0	210.0	34.23
17	0.2	0.146	0.03	20.0	20.0	40.0	150.0	210.0	30.66
18	0.2	0.130	0.03	20.0	20.0	40.0	150.0	210.0	27.30
19	0.2	0.116	0.02	20.0	20.0	40.0	150.0	210.0	24.36
20	—	0.104	—	20.0	20.0	40.0	150.0	210.0	21.84
Total	22.0	—	13.07	—	160.0	320.0	1200.0	1680.0	273.76

[a]All dollar values are in millions of dollars.
[b]Assumes a 12% discount rate.
From Bouis (1999).

Table XII

Sensitivity of Benefit-Cost Ratio to Alternative Cost and Benefit Assumptions

Assumptions regarding central research costs	Assumptions regarding magnitude of benefits	Benefit-cost ratio: Benefits applied in years 11–20 as shown in Table XI	Benefit-cost ratio: Benefits applied in years 16–25 with 5 years additional research costs in Turkey[a]
All central research costs applied to Turkey	Benefits as shown in Table XI	20.9	7.8[b]
	150 kg increment in yield and 40 kg seed savings	11.5	4.3
	Only human nutrition benefit	2.0	0.7
One-tenth of central research costs applied to Turkey	Benefits as shown in Table XI	47.3	14.6
	150 kg increment in yield and 40 kg seed savings	25.9	8.0
	Only human nutrition benefit	4.5	1.4

[a] $10 million increase in current costs in years 10–14 ($2.6 million increase in present value cost), although present values of maintenance breeding costs declines to $0.24 million.

[b] Present value of benefits declines to $120.1 million from $273.8 million in Table XI.

From Bouis (1999).

of these assumptions. Specifically, benefit-cost ratios are calculated assuming instead that: (i) 10% of central costs are apportioned to Turkey, (ii) agronomic benefits are reduced by half over those assumed in Table XI, (iii) only nutrition benefits are realized (no agronomic benefits), and (iv) an additional 5 years and $10 million in costs are required to realize agronomic and human nutrition benefits.

Under the more realistic assumption that only 10% of central costs are applied to Turkey and maintaining the benefits structure applied in Table XI intact, the benefit-cost ratio approaches the extremely high value of 50, which compares well with the benefit claimed by the World Bank for supplementation and fortification programs directed at micronutrient malnutrition.[11]

Delaying benefits by 5 years (and increasing costs) reduces benefit-cost ratios by a factor of about three. Still benefit-cost ratios remain at high levels, except in the extreme case where there are only human nutrition (and no agronomic) benefits and the realization of these benefits is pushed back to years 16–20.

Does this mean that a plant breeding strategy directed at improving human nutrition depends critically on the coexistence of agronomic benefits? Certainly not. Benefit-cost ratios for scenarios involving only benefits to human nutrition in Table XII reasonably could be multiplied by several multiples for the following reasons:

1. *Size of population affected.* Zinc-dense varieties are assumed to reach 1 out of 3 persons in Turkey (ignoring any population growth). Some agricultural research could and should be directed at incorporating Zn-density into high-yielding wheat varieties grown in parts of the country where Zn-deficient soils are not a problem. This would expand the population benefiting from improved Zn intakes.

Moreover, Turkey is not a large country in terms of population. Turkey was selected for these calculations because of the availability of reasonably hard numbers for undertaking estimates of agronomic benefits (see Cakmak, 1999). For more populous countries (e.g., India), the underlying research costs are not necessarily substantially higher, but the benefits may accrue to a much larger number of people.

2. *Size of benefit per person.* According to World Bank estimates cited earlier, elimination of Fe, Zn, and vitamin A deficiency is worth an annual benefit of about $7 per nutrient per capita. A figure of $1 per capita has been used here for Zn. There is no specific reason for lowering the benefit by a factor of seven other than a methodological strategy of undertaking conservative estimates of benefits. The daily "dosage" of Zn supplied through nutritionally-improved staple grains will be lower certainly than could be supplied through supplementation or perhaps commercial fortification, but as yet no Zn supplementation or fortification programs

[11]Turkey accounts for about 10% of wheat area harvested in Asia (FAO, 1995).

are in operation in developing countries. On the other hand, once Zn-rich varieties enter the food production and marketing system they will provide the enhanced dosage day in and day out for an entire lifetime.

This last point highlights an essential difference between investments in standard fortification programs and fortification through plant breeding strategies. Standard fortification programs must be sustained at the same level of funding year after year. If investments are not sustained, benefits disappear. Such investments apply to a single geographical area such as a nation-state. By contrast, investments in research in plant breeding have multiplicative effects—benefits may accrue to a number of countries. Moreover, benefits are sustainable—benefits from breeding advances typically do not disappear after initial investments and research are successful, as long as an effective domestic agricultural research infrastructure is maintained. Finally, these benefits are economic only and do not take into account improved health per se and quality of life, nor the potential of the latter to lower population growth rate.

IX. CONCLUSIONS

Using a plant breeding approach to address micronutrient malnutrition would provide public officials a new "tool" to use in combating diet-related disease in developing countries. As supported by evidence presented in this monograph, this strategy is economical and sustainable. We urge the world's agriculturists, nutritionists, healthcare officials and policy makers to support adoption of this powerful strategy to help eliminate "hidden hunger" from the poor in developing nations so that donor organizations will provide the resources needed to move this strategy forward.

The breeding of nutrient-dense staple foods that give resource-poor populations a better-balanced nutritional base to their diet is feasible from a plant breeding perspective. Results so far indicate that the breeding parameters are not difficult and are highly likely to be cost-effective. The following points are seminal:

- Adequate genetic variation in concentrations of β-carotene, other functional carotenoids, iron, zinc, and other minerals exists in the major germplasm banks to justify selection.
- The micronutrient-density traits are stable across environments.
- In all crops studied, it is possible to combine the high micronutrient-density trait with high yield, unlike protein content and yield that may be negatively correlated.
- The genetic control is simple enough to make breeding economic.
- It will be possible to improve the content of several limiting micronutrients together, thus pushing populations towards nutritional balance.

- High nutrient density not only can benefit the consumer but also produce more vigorous seedlings in the next generation.
- Bioavailability of the extra nutrient in elite breeding lines is high for rats and where the density is high enough for the test, also to human colon cell lines. Tests on human populations are now a high priority.

Moreover, from an economic perspective, the combining of benefits for human nutrition and agricultural productivity, resulting from breeding staple food crops which are more efficient in the uptake of trace minerals from the soil and which load more trace minerals into their seeds, results in extremely high *ex ante* estimates of benefit-costs ratios for investments in agricultural research in this area. This finding derives from the confluence of several complementary factors:

1. Rates of micronutrient malnutrition are high in developing countries, as are the consequent costs to human welfare and economic productivity.
2. Because staple foods are eaten in large quantities every day by the malnourished poor, delivery of enriched staple foods (fortified by the plants themselves during growth) can rely on existing consumer behavior.
3. A significant percentage of the soils in which these staple foods are grown is deficient in one or more of these nutrients, which has kept crop yields low. In general, these soils in fact contain high amounts of trace minerals (sufficient for hundreds of crops). However, because of chemical binding to soil particles, these trace minerals are poorly available to the staple crop varieties under present use by farmers.
4. Adoption and spread of nutritionally improved cultivars by farmers can rely on profit incentives, either because of agronomic advantages on micronutrient-deficient soils or because the nutritional improvements are incorporated in the most productive cultivars being released by agricultural research stations.
5. Benefits to relatively small investments in agricultural research may be disseminated widely, potentially accruing to hundreds of millions of people and hectares of cropping lands.
6. Benefits from breeding advances, derived from initial, fixed costs, typically do not involve high recurring costs, and thus tend to be highly sustainable as long as an effective domestic agricultural research infrastructure is maintained.

In treating Fe deficiency in developing countries, Yip (1994) argues that if prevalence rates are above 25%, the best approach is to develop programs to improve the Fe status of the entire population. In such situations, which for preschoolers and women in developing countries are the rule rather than the exception, this is cheaper than screening for Fe-deficient individuals. By increasing the Fe content of food staples through plant breeding, the entire Fe status distribution curve can be shifted to the right, so that targeting a subsequently smaller group of Fe-deficient persons could become feasible. On the other hand, since the cultivars under development probably contain phytate-bound Fe of plant origins, their dissemination can be expected not to increase the risk of Fe toxicity that may exist in a very low percentage of wealthy individuals in the population.

Unfortunately, much less is known about the prevalence of Zn deficiency in developing countries, or about the distribution curve for biochemical indicators of Zn status. Even less is known about the cost of interventions for the prevention and control of Zn deficiency simply because wide-scale testing of possible interventions awaits conclusion of the debate over whether Zn deficiency should be regarded as a major public health problem. Certainly, plant breeding is an option that should be exploited as quickly as possible to reduce Zn deficiency.

A plant breeding strategy, if successful, will not eliminate the need for supplementation, fortification, dietary diversification, and disease reduction programs in the future to combat micronutrient malnutrition. Nevertheless, this strategy does hold great promise for significantly reducing recurrent expenditures required for these higher-cost, short-run programs by significantly reducing the numbers of people requiring treatment. Cost is not a key issue in the decision to pursue a plant breeding strategy to improve human nutrition. A relatively modest level of resources is required and the potential payoff is quite high.

Rather, the two key issues are: (i) will staple varieties with mineral-dense seeds be widely adopted by farmers in developing countries, either because of agronomic advantages on trace mineral-deficient soils or through this characteristic being bred into the highest-yielding varieties? and (ii) will the additional nutrients contained in the seeds be of a sufficient magnitude and sufficiently bioavailable so as to have an appreciable impact on micronutrient status and health?

References

Allaway, W. H. (1986). Soil-plant-animal and human interrelationships in trace element nutrition. pp. 465–488. In: Trace Elements in Human and Animal Nutrition. W. Mertz, (Ed.) Academic Press, Orlando, FL.

Andrews, S. C., Arosio, P., Bottke, W., Briat, J.-F., Von Darl, M., Harrison, P. M., Laulhère, J.-P., Levi, S., Lobreaux, S., and Yewdall, S. J. (1992). Structure, function, and evolution of ferritins. *J. Inorg. Biochem.* **47,** 161–174.

Anonymous. (1994). The challenge of dietary deficiencies of vitamins and minerals. In: Enriching Lives: Overcoming Vitamin and Mineral Malnutrition in Developing Countries. pp. 6–13. World Bank, Washington D.C.

Arredondo-Peter, R., Hargrove, M. S., and Sarath, G. (1997). Rice hemoglobins. Gene cloning, analysis, and 02-binding kinetics of a recombinant. *Plant Physiol.* **115,** 1259–1266.

Ascher, J. S. (1994). Phosphorus and manganese seed coatings for crop growth and yield. Ph. D. thesis, University of New England, Armidale.

Ashmead, D., and Christy, H. (1985). Factors interfering with intestinal absorption of minerals. *An. Nutr. Health* **40,** 10–13.

Barisani, D., Green, R. M., and Gollan, J. L. (1996). Genetic hemochromatosis: Pathogenesis, diagnosis, and therapy. *Dig. Dis.* **14,** 304–315.

Beard, J. L., Burton, J. W., and Theil, E. C. (1996). Purified ferritin and soybean meal can be sources of iron for treating iron deficiency in rats. *J. Nutr.* **126,** 154–160.

Benito, P., and Miller, D. (1998). Iron absorption and bioavailability: An updated review. *Nutr. Res.* **18,** 581–603.

Bouis, H. E. (1994). The effect of income on demand for food in poor countries: Are our databases giving us reliable estimates? *J. Dev. Econ.* **44**(1), 199–226.

Bouis, H. E. (1996). A food demand system based on demand for characteristics: If there is 'curvature' in the Slutsky matrix, what do the curves look like and why? *J. Dev. Econ.* **51**(2), 239–266.

Bouis, H. E. (1999). Economics of enhanced micronutrient density in food staples. *Field Crops Res.* **60,** 165–173.

Bouis, H. E., and Novenario-Reese, M. J. (1997). The determinants of demand for micronutrients: An analysis of rural households in Bangladesh. FCND Discussion Paper. International Food Policy Research Institute, Washington, D.C.

Bouis, H. E., and Haddad, L. J. (1990). Agricultural Commercialization, Nutrition, and the Rural Poor: A study of Philippine Farm Households. Lynne Rienner Publishers, Boulder, CO.

Briat, J. F. (1996). Roles of ferritin in plants. *J. Plant Nutr.* **19,** 1331–1342.

Brown, K. H. (1998). Effect of infections on plasma zinc concentration and implications for zinc status assessment in low-income countries. *Am. J. Clin. Nutr.* **68** (Suppl. S), 425S–429S.

Brown, L. R., and Flavin, C. (1999). A new economy for a new century. *In* "State of the World." L. R. Brown, C. Flavin, H. F. French, and L. Starke, (Eds.) pp. 3–21. W. W. Norton and Company, NY.

Brunson, A. M., and Quackenbush, F. W. (1962). Breeding corn with high pro-vitamin A in the grain. *Crop Sci.* **2,** 344–347.

Cakmak, I. (1999). Zinc deficiency as a practical problem in plant and human nutrition in Turkey: A NATO-Science for Stability Project. *Field Crops Res.* **60,** 175–188.

Clark, L. C., Combs, G. F., Turnbull, W. B., Slate, E. H., Chalker, D. K., Chow, J., Davis, L. S., Glover, R. A., Graham, G. F., Gross, E. G., Krongrad, A., Lesher, J. L., Park, H. K., Sanders, B. B., Smith, C. L., Taylor, J. R., and The Nutritional Prevention of Cancer Study Group. (1996). Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: a randomized controlled trial. *J. Am. Med. Assoc.,* **276** (24), 1957–1963.

Combs, G. F., Jr. (1995). Emphasizing fruits and vegetables in American diets: implications for vitamin and mineral intakes. *In* "Division of Nutritional Sciences, Conference." Anonymous (Ed.), pp. 52–57. Cornell University, Ithaca, NY.

Combs, G. F., Jr. (1998). "The Vitamins. Fundamental Aspects in Nutrition and Health." Academic Press, N.Y. 618 p.

Combs, G. F., Jr., Welch, R. M., and Duxbury, J. M. (1997). Food systems for improved health: linking agricultural production to human nutrition. *Euro. J. Clin. Nutr.* **51,** S32-S33.

Combs, G. F., Welch, R. M., Duxbury, J. M., Uphoff, N. T., and Nesheim, M. C. (1996). "Food-Based Approaches to Preventing Micronutrient Malnutrition: An International Research Agenda." Cornell International Institute for Food, Agriculture, and Development, Cornell University, Ithaca, NY.

Cousins, R. J., and Hempe, J. (1990). Zinc. *In* "Present Knowledge in Nutrition." M. L. Brown, (Ed.), pp. 251–260. Nutrition Foundation, Washington, D.C.

Crosbie, J., Longnecker, N. E., Davies, F., and Robson, A. D. (1993). Effects of seed manganese concentration on lupin emergence. *In* "Plant Nutrition—From Genetic Engineering to Field Practice." N. J. Barrow (Ed), pp. 665–668. Kluwer Academic Publishers, Dordrecht.

Dallman, P. R. (1990). Iron. *In* "Present Knowledge in Nutrition." M. L. Brown (Ed.), pp. 241–250. International Life Sciences Institute, Nutrition Foundation, Washington, D.C.

Duff, S. M. G., Guy, P. A., Nie, X. Z., Durnin, D. C., and Hill, R. D. (1998). Haemoglobin expression in germinating barley. *Seed Sci. Res.* **8,** 431–436.

Epstein, E. (1972). Mineral Nutrition of Plants: Principles and Perspectives. Wiley, New York.

Erdman, J. W. Jr., and Poneros-Schneier, A. (1989). Phytic acid interactions with divalent cations in foods and in the gastrointestinal tract. *Adv. Exp. Med. Biol.* **249,** 161–171.

Ertl, D. S., Young, K. A., and Raboy, V. (1998). Plant genetic approaches to phosphorus management in agricultural production. *J. Environ. Qual.* **27,** 299–304.

Fairweather-Tait, S., and Hurrell, R. F. (1996). Bioavailability of minerals and trace elements. *Nutr. Res. Rev.* **9,** 295–324.

Fehr, W. R. (1982). Control of iron deficiency chlorosis in soybeans by plant breeding. *J. Plant Nutr.* **5,** 611–621.

Fischer, P. R., Rahman, A., Cimma, J. P., Kyaw-Myint, T. O., Kabir, A. R. M. L., Talukder, K., Hassan, N., Manaster, B. J., Staab, D. B., Duxbury, J. M., Welch, R. M., Meisner, C. A., Haque, S., and Combs, G. F. Jr. (1998). Nutritional rickets without vitamin D deficiency in Bangladesh. *Pediatrics* **45,** 291–293.

Food and Agricultural Organization of the United Nations (FAO). (1995). FAO Stat-PC, Trade and Production Domains, Version 3.0 (computer disk), Rome.

Food and Agricultural Organization of the United Nations (FAO). (1997). *Food Outlook.* No. 5/6, May/June, Rome.

Garcia-Casal, M. N., and Layrisse, M. (1999). Vitamin A and β-carotene can improve nonheme iron absorption from rice, wheat and corn by humans. *FASEB J.* **13,** A243.

Garcia-Casal, M. N., Layrisse, M., Solano, L., Arguello, F., Llovera, D., Ramírez, J., Leets, I., and Tropper, E. (1998). Vitamin A and β-carotene can improve nonheme iron absorption from rice, wheat and corn by humans. *J. Nutr.* **128,** 646–650.

Gerloff, G. C., and Gabelman, W. H. (1983). Genetic basis of inorganic plant nutrition. *In* "Encyclopedia of Plant Physiology. New Series." A. Lauchli and R. L. Bieleski (Eds.), Vol. 15B. pp. 453–480. Springer-Verlag, Berlin.

Gibson, R. S., and Ferguson, E. L. (1998). Assessment of dietary zinc in a population. *Am. J. Clin. Nutr.* **68,** 430S–434S.

Gibson, R. S. (1994). Zinc nutrition in developing countries. *Nutr. Res. Rev.* **7,** 151–173.

Gittinger, J. P. (1982). Economic Analysis of Agricultural Projects. EDI Series in Economic Development. The Johns Hopkins University Press, Baltimore and London.

Gopalan, C. (1999). The changing epidemiology of malnutrition in a developing society. The effect of unforeseen factors. *Bull. Nutr. Found. India* **20,** 1–5.

Gordon, D. T., and Godber, J. S. (1988). The enhancement of nonheme iron bioavailability by beef protein in the rat. *J. Nutr.* **119,** 446–452.

Goto, F., Yoshihara, T., Shigemoto, N., Toki, S., and Takaiwa, F. (1999). Iron fortification of rice seeds by the soybean ferritin gene. *Nature Biotech.* **17,** 282–286.

Graham, R. D., and Welch, R. M. (1996). Breeding for staple food crops with high micronutrient density. Agricultural Strategies for Micronutrients. Working Paper 3. pp. 1–72. International Food Policy Research Institute, Washington, D.C.

Graham, R. D. (1984). Breeding for nutritional characteristics in cereals. *Adv. Plant Nutr.* **1,** 57–102.

Graham, R. D. (1990). Breeding wheats for tolerance to micronutrient deficient soil: present status and priorities. *In* "Wheat for the Nontraditional Warm Areas." D. A. Saunders (Ed.), pp. 315–332. CIMMYT, Mexico D. F.

Graham, R. D., Anderson, G. D., and Ascher, J. S. (1981). Absorption of copper by wheat, rye and some hybrid genotypes. *J. Plant Nutr.* **3,** 679–686.

Graham, R. D., Ascher, J. S., Ellis, P. A. E., and Shepherd, K. W. (1987). Transfer to wheat of the copper efficiency factor carried on rye chromosome arm 5RL. *Plant Soil* **99,** 107–114.

Graham, R. D., Senadhira, D., and Ortiz-Monasterio, I. (1997). A strategy for breeding staple-food crops with high micronutrient density. *Soil Sci. Plant Nutr.* **43,** 1153–1157.

Graham, R. D., Senadhira, D., Beebe, S. E., Iglesias, C., and Ortiz-Monasterio, I. (1999). Breeding for micronutrient density in edible portions of staple food crops: Conventional approaches. *Field Crops Res.* **60,** 57–80.

Graham, R. D., Ascher, J. S., and Hynes, S. C. (1992). Selecting zinc-efficient cereal genotypes for soils of low zinc status. *Plant Soil* **146,** 241–250.

Grewal, H. S., Graham, R. D., and Howes, N. (1997). Bioassays and molecular markers for zinc efficiency in cereals. p 29. Annu. Rep., CRC Mol. Plant Breeding, University of Adelaide.

Gurley, W. H., and Giddens, J. (1969). Factors affecting uptake, yield response, and carryover of molybdenum in soybean seed. *Agron. J.* **61,** 7–9.

Hallberg, L., Brune, M., and Rossander, L. (1989). Iron absorption in man: Ascorbic acid and dose-dependent inhibition of phytate. *Am. J. Clin. Nutr.* **49,** 140–144.

Hambidge, K. M., Casey, C. E., and Krebs, N. F. (1986). Zinc. *In* "Trace Elements in Human and Animal Nutrition." Vol. 2, W. Mertz (Ed.), pp. 1–137. Academic Press, Inc., NY.

Harland, B. F., and Morris, E. R. (1995). Phytate: A good or a bad food component. *Nutr. Res.* **15,** 733–754.

Harland, B. F., and Narula, G. (1999). Food phytate and its hydrolysis products. *Nutr. Res.* **19,** 947–961.

Hartwig, E. E., Jones, W. F., and Kilen, T. C. (1991). Identification and inheritance of inefficient zinc absorption in soybean. *Crop Sci.* **31,** 61–63.

Hill, R. D. (1998). What are hemoglobins doing in plants? *Can. J. Bot.* **76,** 707–712.

House, W. A. (1999). Trace element bioavailability as exemplified by iron and zinc. *Field Crops Res.* **60,** 115–141.

House, W. A., Van Campen, D. R., and Welch, R. M. (1996). Influence of dietary sulfur-containing amino acids on the bioavailability to rats of zinc in corn kernels. *Nutr. Res.* **16,** 225–235.

House, W. A., Van Campen, D. R., and Welch, R. M. (1997). Dietary methionine status and its relation to the bioavailability to rats of zinc in corn kernels with varying methionine content. *Nutr. Res.* **17,** 65–76.

House, W. A., and Welch, R. M. (1989). Bioavailability of and interactions between zinc and selenium in rats fed wheat grain intrinsically labeled with ^{65}Zn and ^{75}Se. *J. Nutr.* **119,** 916–921.

Hunt, J. R. (1996). Bioavailability algorithms in setting recommended dietary allowances: Lessons from iron, applications to zinc. *J. Nutr.* **126** (9 Suppl.), 2345S–2353S.

Hunt, J. R., Gallagher, S. K., and Johnson, L. K. (1994). Effect of ascorbic acid on apparent iron absorption by women with low iron stores. *Am. J. Clin. Nutr.* **59,** 1381–1385.

Hurrell, R. F. (1997). Bioavailability of iron. *Euro. J. Clin. Nutr.* **51** (Suppl. 1), S4–S8.

Iglesias, C., Mayer, J., Chavez, L., and Calle, F. (1997). Genetic potential and stability of carotene content of cassava roots. *Euphytica* **94,** 367–373.

IRRI. (1979). Annual Report, International Rice Research Institute, Los Banos, The Philippines.

Kaji, M., Gotoh, M., Takagi, Y., Masuda, H., Kimura, Y., and Uenoyama, Y. (1998). Studies to determine the usefulness of the zinc clearance test to diagnose marginal zinc deficiency and the effects of oral zinc supplementation for short children. *J. Am. College Nutr.* **17,** 388–391.

Kapsokefalou, M., and Miller, D. D. (1995). Iron speciation intestinal contents of rats fed meals composed of meat and nonmeat sources of protein and fat. *Food Chem.* **52,** 47–56.

Khachik, F., Bernstein, P. S., Squires, A., and Rosser, J. M. (1999). Identification of carotenoids and related metabolites in frog retina and liver: a useful non-primate model for studying the physiological role of macular carotenoids. Proc. 12th Int. Carotenoid Symp., Cairns, Aust. July 19–24.

Layrisse, M., Garcia-Casal, M. N., Solano, L., Baron, M. A., Arguello, F., Llovera, D., Ramírez, J., Leets, I., and Tropper, E. (1997). The role of vitamin A on the inhibitors of nonheme iron absorption: Preliminary results. *J. Nutr. Biochem.* **8,** 61–67.

Layrisse, M., García-Casal, M. N., Solano, L., Baron, M. A., Arguello, F., Llovera, D., Ramírez, J., Leets, I., and Tropper, E. (1998). Vitamin A reduces the inhibition of iron absorption by phytates and pholyphenols. *Food Nutr. Bull.* **19,** 3–5.

Longnecker, N. E., Graham, R. D., and Marcar, N. E. (1988). The effect of seed manganese on barley

(*Hordeum vulgare* L.) growth and yield. *In* "International Symposium on Manganese in Soils and Plants: Contributed Papers." M. J. Webb, R. O. Nable, R. D. Graham, and R. J. Hannam (Eds.), pp. 47–50. Manganese Symposium, 1988 Inc., Adelaide.

Longnecker, N. E., Marcar, N. E., and Graham, R. D. (1991). Increased manganese content of barley seeds can increase grain yield in manganese deficient conditions. *Aust. J. Agric. Res.* **42,** 1065–1074.

Lott, J. N. A. (1984). Accumulation of seed reserves of phosphorus and other minerals. *In* "Seed Physiology," Vol. I. Development, D. R. Murray (Ed.). pp. 139–166. Academic Press, Sydney, New York, London.

Maberly, G. F., Trowbridge, F. L., Yip, R., Sullivan, K. M., and West, C. E. (1994). Programs against micronutrient malnutrition: ending hidden hunger. *Ann. Rev. Public Health* **15,** 277–301.

Majumder, N. D., Rakshit, S. C., and Borthakur, D. N. (1990). Genetic effects on uptake of selected nutrients in some rice (*Oryza sativa* L.) varieties in phosphorus deficient soil. *Plant Soil* **123,** 117–120.

Marcar, N. E., and Graham, R. D. (1986). Effect of seed manganese content on growth of wheat (*Triticum aestivum*) under manganese deficiency. *Plant Soil* **96:** 165–173.

Marschner, H. (1995). "Mineral Nutrition of Higher Plants," 2nd ed. Academic Press, London.

Matzke, H. J. (1998). Impact of processing on bioavailability examples of minerals in foods. *Trends Food Sci. Tech.* **9,** 320–327.

Mazzolini, A. P., and Legge, G. J. F. (1981). The distribution of trace elements in mature wheat seed using the Melbourne proton microprobe. *Nuclear Instr. Meth.* **191,** 583–589.

McCarthy, K. W., Longnecker, N. E., Sparrow, D. H. B., and Graham, R. D. (1988). Inheritance of manganese efficiency in barley (*Hordeum vulgare* L.). *In* "International Symposium on Manganese in Soils and Plants: Contributed Papers." M. J. Webb, R. O. Nable, R. D. Graham, and R. J. Hannam (Eds.). pp. 121–122. Manganese Symposium, 1988 Inc., Adelaide.

Mendoza, C., Viteri, F. E., Lonnerdal, B., Young, K. A., Raboy, V., and Brown, K. H. (1998). Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. *Am. J. Clin. Nutr.* **68,** 1123–1127.

Michaelsen, K. F., and Friis, H. (1998). Complementary feeding: A global perspective. *Nutrition* **14,** 763–766.

Morris, E. R. (1986). Phytate and dietary mineral bioavailability. *In* "Phytic Acid: Chemistry and Applications," E. Graf (Ed.), pp. 57–76. Pilatus Press, Minneapolis.

Morris, E. R., and Ellis, R. (1982). Phytate, wheat bran, and bioavailability of dietary iron. *In* "Nutritional Bioavailability of Iron," C. Kies (Ed.), pp. 121–141. American Chemical Society, Washington, D.C.

Moussavi-Nik, M., Rengel, Z., Hollamby, G. J., and Ascher, J. S. (1997). Seed manganese (Mn) content is more important than Mn fertilisation for wheat growth under Mn-deficient conditions. *In* "Plant Nutrition for Sustainable Food Production and Environment." T. Ando, K. Fujita, T. Mae, H. Matsumoto, S. Mori, and J. Sekiya (Eds.), pp. 267–268. Kluwer Academic Publishers, Dordrecht.

Mulvihill, B., and Morrissey, P. A. (1998a). An investigation of factors influencing the bioavailability of non-haem iron from meat systems. *Irish J. Agric. Food Res.* **37,** 219–226.

Mulvihill, B., and Morrissey, P. A. (1998b). Influence of the sulphhydryl content of animal proteins on in vitro bioavailability of non-haem iron. *Food Chem.* **61,** 1–7.

Ogawa, M., Tanaka, K., and Kasai, A. (1979). Energy-dispersive x-ray analysis of phytin globoids in aleurone particles of developing rice grains. *Soil Sci. Plant Nutr.* **25,** 437–438.

Ortiz-Monasterio, I. (1998). "CGIAR Micronutrients Project," Update No 3. p5. International Food Policy Research Institute, Washington, D.C.

Paik, H. Y., Joung, H., Lee, J. Y., Lee, H. K., King, J. C., and Keen, C. L. (1999). Serum extracellular superoxide dismutase activity as an indicator of zinc status in humans. *Biol. Trace Element Res.* **69,** 45–57.

Pallauf, J., and Rimbach, G. (1997). Nutritional significance of phytic acid and phytase. *Arch. Animal Nutr.* **50,** 301–319.

Pennington, J. A. T., and Young, B. (1990). Iron, zinc, copper, manganese, selenium, and iodine in foods from the United States total diet study. *J. Food Compos. Anal.* **3,** 166–184.

Phillips, R. L., and McLure, B. A. (1985). Elevated protein-bound methionine in seeds of a maize line resistant to lysine plus threonine. *Cereal Chem.* **62,** 213–218.

Pinstrup-Andersen, P. (1999). "Food Policy Research for Developing Countries. Emerging Issues and Unfinished Business." International Food Policy Research Institute, Washington, D.C.

Politz, M. L., and Clydesdale, F. M. (1988). Effect of enzymatic digestion, pH and molecular weight on the iron solubilizing properties of chicken muscle. *J. Food Sci.* **53,** 1081–1090.

Ponnamperuma, F. N. (1976). Screening rice for tolerance to mineral stresses. *In* "Plant Adaptation to Mineral Stress in Problem Soils." M. J. Wright. (Ed.) pp. 341–353. Cornell University Agricultural Experiment Station, Ithaca, N.Y.

Rao, B. S. N. (1994). Methods for the determination of bioavailability of trace metals: A critical evaluation. *J. Food Sci. Tech.* **31,** 353–361.

Rao, V. S., Gangwar, M. S., and Rathore, V. S. (1977). Genotypic variation in distribution of total and labelled zinc and availability of zinc (A and L values) to soybeans grown in mollisol. *J. Agric. Sci.* **8,** 417–420.

Reddy, M. B., and Cook, J. D. (1991). Assessment of dietary determinants of nonheme-iron absorption in humans and rats. *Am. J. Clin. Nutr.* **54,** 723–728.

Rengel, Z., and Graham, R. D. (1995). Importance of seed Zn content for wheat growth on Zn-deficient soil: I. Vegetative growth. *Plant Soil* **173,** 259–266.

Ripperger, H., and Schreiber, K. (1982). Nicotianamine and analogous amino acids, endogenous iron carriers in higher plants. *Heterocycles* **17,** 447–461.

Rose, I. A., Felton, W. L., and Banke, L. W. (1981). Response of four soybean varieties to foliar zinc fertilizer. *Aust. J. Exp. Agric. Anim. Husb.* **21,** 236–240.

Rosser, J. M., Khachik F., and Graham R. D. (1999). "Distribution of lutein and zeaxanthin and related geometrical isomers in fruits, vegetables, wheat, and pasta products." Abstr. 12th International Symposium on Carotenoids, Cairns, Australia. July 19–24.

Sakamoto, K., Vucenik, I., and Shamsuddin, A. M. (1993). [^{3}H]Phytic acid (inositol hexaphosphate) is absorbed and distributed to various tissues in rats. *J. Nutr.* **123,** 713–720.

Salunkhe, D. K., and Desai, B. B. (1988). Effects of agricultural practices, handling, processing, and strorage of vegetables. *In* Nutritional Evaluation of Food Processing." E. Karmas, and R. S. Harris (Eds.), pp. 23–71. Avi Book, Van Nostrand Reinhold Co., NY.

Saxena, S . C., and Chandel, A. S. (1992). Effect of zinc fertilization on different varieties of soybean (*Glycine max*). *Indian J. Agric. Sci.* **62,** 695–697.

Senadhira, D., and Graham, R. D. (1999). Genetic variation in iron and zinc concentrations in brown rice. *Micronutr. Agric.* No 3, 10–12.

Siegenberg, D., Baynes, R. D., Bothwell, T. H., MacFarlane, B. J., Lamparelli, R. D., Car, N. G., MacPhail, P., Schmidt, U., Tal, A., and Mayet, F. (1991). Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *Am. J. Clin. Nutr.* **53,** 537–541.

Simon, P. W. (1992). Genetic improvement of vegetable carotene content. *In* "Biotechnology and Nutrition. Proceedings of the Third International Symposium." D. D. Bills, and S-Y. Kung (Eds.), pp. 291–314. Butterworth-Heinemann, Boston.

Sowa, A. W., Duff, S. M. G., Guy, P. A., and Hill, R. D. (1998). Altering hemoglobin levels changes energy status in maize cells under hypoxia. *Proc. Natl. Acad. Sci.* **95,** 10317–10321.

Subramanian, S., and Deaton, A. (1996). The demand for food and calories. *J. Political Econ.* **104**(1), 133–162.

Sugiura, S. H., and Raboy, V. (1999). Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (Oncohynchus mykiss). *Aquaculture* **170,** 285–296.

Taylor, P. G., Martínez-Torres, C., and Romano, E. L. (1986). The effect of cysteine-containing peptides released during meat digestion on iron absorption in humans. *Am. J. Clin. Nutr.* **43,** 68–71.

Theil, E. C., Burton, J. W., and Beard, J. L. (1997). A sustainable solution for dietary iron deficiency through plant biotechnology and breeding to increase seed ferritin control. *Euro. J. Biochem.* **51,** S28-S31.

Thompson, B. D., Bell, R. W., and Bolland, M. D. A. (1990). Low seed phosphorus concentration depresses early growth and nodulation of narrow-leafed lupin (*Lupinus angustifolius* cv. Gungurru). *J. Plant Nutr.* **14,** 1355–1367.

Tuntawiroon, M., Sritongkul, N., Rossander-Hulten, L., Pleehachinda, R., Suwanik, R., Brune, M., and Hallberg, L. (1999). Rice and iron absorption in man. *Euro. J. Clin. Nutr.* **44,** 489–497.

Underwood, B. A. (1998). From research to global reality: the micronutrient story. *J. Nutr.* **128,** 145–151.

United Nations Administrative Committee on Coordination—Subcommittee on Nutrition. (1992). Second Report on the World Nutrition Situation. Vol. I. Global and Regional Results. pp. 1–80. Geneva, Switzerland.

United Nations. (1998). World population growth from year 0 to 2050. World Population Prospects: The 1998 Revision, United Nations, NY.

Uvin, P. (1994). The state of world hunger. *Nutr. Rev.* **52,** 151–161.

Van Campen, D. R., and Glahn, R. P. (1999). Micronutrient bioavailability techniques: Accuracy, problems and limitations. *Field Crops Res.* **60,** 93–113.

Weir, R. G., and Hudson, A. (1966). Molybdenum deficiency in maize in relation to seed reserves. *Aust. J. Exp. Agric. Anim. Husb.* **6,** 35–41.

Weiss, M. G. (1943). Inheritance and physiology of efficiency in iron utilisation in soybeans. *Genetics* **28,** 253–268.

Welch, R. M. (1995). Micronutrient nutrition of plants. *Crit. Rev. Plant Sci.,* **14,** 49–82.

Welch, R. M. (1993). Zinc concentrations and forms in plants for humans and animals. *In* "Zinc in Soils and Plants." A. D. Robson, (Ed.) pp. 183–195. Kluwer Academic Publishers, Dordrecht.

Welch, R. M., and House, W. A. (1984). Factors affecting the bioavailability of mineral nutrients in plant foods. *In* "Crops as Sources of Nutrients for Humans." R. M. Welch, and W. H. Gabelman (Eds.), pp. 37–54. American Society of Agronomy, Madison, WI.

Welch, R. M., and House, W. A. (1995). Meat factors in animal products that enhance iron and zinc bioavailability: Implications for improving the nutritional quality of seeds and grains. Cornell Nutrition Conference for Feed Manufacturers. pp. 58–66. Department of Animal Science and Division of Nutrition, Cornell University Agricultural Experiment Station, Ithaca, NY.

Welch, R. M., House, W. A., and Allaway, W. H. (1974). Availability of zinc from pea seeds to rats. *J. Nutr.* **104,** 733–740.

Welch, R. M. (1986). Effects of nutrient deficiencies on seed production and quality. *Adv. Plant Nutr.* **2,** 205–247.

Welch, R. M., and Graham, R. D. (1999). A new paradigm for world agriculture: Meeting human needs; productive, sustainable, nutritious. *Field Crops Res.* **60,** 1–10.

Welch, R. M., Combs, G. F., Jr., and Duxbury, J. M. (1997). Toward a "greener" revolution. *Issues Sci. Technol.* **14,** 50–58.

WHO. (1996). Trace Elements in Human Nutrition and Health. World Health Organization, Geneva.

Wienk, K. J. H., Marx, J. J. M., and Beynen, A. C. (1999). The concept of iron bioavailability and its assessment. *Euro. J. Clin. Nutr.* **38,** 51–75.

World Bank. (1994). Enriching Lives: Overcoming Vitamin and Mineral Malnutrition in Developing Countries. Development in Practice Series. World Bank, Washington, D.C.

World Health Organization. (1996). World Health pp. 1–343. Organization, Geneva.
World Health Organization. (1999). *http://www.who.int/nut/malnutritionworldwide.htm* Malnutrition Worldwide. World Health Organization, Geneva.
Ye, X., Al-Babili, S., Klöti, A., Zhang, J., Lucca, P., Beyer, P. and Potrykus, I. (2000). Engineering the pro-vitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* **287,** 303–305.
Yilmaz, A., Ekiz, H., Gultekin, I., Torun, B., Karanlik, S., and Cakmak, I. (1997). Effect of seed zinc content on grain yield and zinc concentration of wheat grown in zinc-deficient calcareous soils. *In* "Plant Nutrition for Sustainable Food Production and Environment." T. Ando, K. Fujita, T. Mae, H. Matsumoto, S. Mori, and J. Sekiya (Eds.), pp. 283–284. Kluwer Academic Publishers, Dordrecht.
Yip, R. (1994). Iron deficiency: contemporary scientific issues and international programmatic approaches. *J. Nutr.* **124,** 1479S–90S.
Zhou, J. R., and Erdman, J. W., Jr. (1995). Phytic acid in health and disease. *Crit. Rev. Food Sci. Nutr.* **35,** 495–508.